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Control of *Parthenium hysterophorus* L. using *Listronotus setosipennis* Hustache (Coleoptera: Curculionidae) in Ethiopia

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ABSTRACT: Laboratory experiments were conducted for fixing optimum dose requirement of the biocontrol agent, *Listronotus setosipennis* Hustache for the control of *Parthenium hysterophorus* L. and the regeneration capacity of the treated weeds under quarantine conditions at Ambo Plant Protection Research Center (APPRC), Ethiopia during 2010-11. The weevils neither feed and stay nor lay eggs on any test plants except parthenium. They were active only on parthenium flowers and leaves during night and hidden in the soil during day times and inactive on other used test plants. The maximum weevils mortality of 20% was recorded on *Bidens pachyloma*, *B. pilosa*, *Lactuca sativa* and *Vicia faba* and no mortality was on *Guizotia scabra* and on *P. hysterophorus*. Complete wilting of parthenium plants was observed when larval population was 25.33, 29.33 and 35 per parthenium plant, >90% wilting was on 20 larvae, >80% wilting was on 14.33 larvae and >50% wilting was on 9.33 larvae after 25 days of its release. Plant height was reduced up to 27% on 14.33 larvae and subsequent larval numbers after 25 days of its release. More than 50% plant biomass was reduced on 25.33 larvae and number of roots and root length was also significantly reduced on treated plants over control. Higher number of larvae i.e. 20, 25.33, 29.33 and 35 are recommended to control parthenium because plants were not regenerated. Even though, this bioagent is safe to release to combat parthenium in quarantine conditions but it should be released in restricted area under field conditions with proper monitoring. Life-table studies of this weevil are needed under field conditions to know the fate of it in the field. © 2011 Association for Advancement of Entomology

KEYWORDS: Host specificity test, *Listronotus setosipennis* Hustache, regeneration capacity, asteraceous plants, Parthenium

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INTRODUCTION

Parthenium, *Parthenium hysterophorus* L. is an herbaceous invasive annual weed believed to be introduced to Ethiopia in 1970s and has been widely distributed in East Africa, India, Australia and in many other countries (Tamado Tenna and Milberg, 2000). Taye Tessema (2002) stated that many cereals, pulses, vegetables and fruit crops were found to be infested by it in Ethiopia. It is causing 40%–97% reduction in sorghum grain yield in eastern Ethiopia (Tamado Tenna, 2001). This weed is also poisonous, pernicious, problematic, allergic and aggressively growing one posing serious threat to human beings and livestock. Stem-boring weevil, *Listronotus setosipennis* Hustache (Coleoptera: Curculionidae) is a 5 mm long and nocturnal which feeds usually on parthenium flowers and sometimes on its leaves and larvae has the ability to kill or prevent further development of parthenium seedlings by boring the stem (Wild *et al.*, 1992). It is promising bioagent in regions with prolonged dry periods and erratic rainfall patterns (Dhileepan, 2003). This weevil was not recorded as a pest of any crop or pasture in South America and it had never been encountered attacking sunflower in northern Argentina (Quintana and Abot, 1987). Detailed host specificity tests showed restricted host-range despite the fact that some feeding and development was recorded on sunflower but is safe bioagent to control parthenium (Wild *et al.*, 1992). Dhileepan (2003) reported that this weevil reduced the plant height by 51%, number of leaves by 78%, flower production by 63% and plant biomass by 54% in glasshouse. *L. setosipennis* was introduced to Australia from Argentina and Brazil in 1982–86 (McFadyen, 1985; Wild *et al.*, 1992) after confirmation of host specificity tests in Brazil (Wild, 1980) and it was established immediately after releases in 1983 (Wild *et al.*, 1992) but its field incidence was low and sporadic (McFadyen, 1992; Dhileepan *et al.*, 1996; Dhileepan and McFadyen, 1997; Dhileepan, 2003). Host specificity test of this weevil in South Africa is still under quarantine conditions which introduced from Santiago, northwestern Argentina (Strathie *et al.*, 2005). So far, scientific evaluation of this weevil was not studied in Ethiopia. Hence, preliminary observations on safety, efficacy and regeneration of wilted parthenium to this weed killer were done under quarantine conditions in Ambo, Ethiopia.

MATERIALS AND METHODS

Host specificity test was conducted at Ambo Plant Protection Research Center (APPRC), Ambo, during 2010–11 under quarantine conditions at temperature 29±3°C and relative humidity 60 ± 5%. The weevil was reared on potted plants kept in cages made of wooden frames with polyethylene sheet on top and nylon mesh on four sides. On ten economically important crops, weeds and forage grasses i.e. *Vernonia galamensis* (oil and varnish purpose), *Lactuca sativa* (salad crop), sunflower (*Helianthus annus*) variety i.e R. Black and Oissia, wheat (*Triticum aestivum*), faba bean (*Vicia faba*) variety i.e. wayyuu, *Bidens pilosa* (medicinal as well as weed), *B. pachyloma* (ornamental in meskal and Ethiopian new year festival), two variety of *Guizotia abyssinica* variety: kuyyuu, shambu (major oil crop of Ethiopia), *Capsicum* sp. *B. ghedoensis* (forage for cattle as well as weed) and three weeds i.e.

TABLE 1. Per cent adult mortality of *L. setosipennis* on test plants and parthenium

Test plants	Adult mortality (%)
Bidens ghedoensis	10abcd
B. pachyloma	20a
B. pilosa	20a
Conyza bonariensis	16.67ab
Capsicum sp.	3.33cd
Flaveria trinervia	13.33abc
Guizotia abyssinica (kuyyuu)	16.67ab
G. abyssinica (shambu)	16.67ab
G. scabra	0d
Helianthus annus (R. Black)	3.33cd
H. annus (Oissia)	3.33cd
Lactuca sativa	20a
Tagetes minuta	6.67bcd
Triticum aestivum	6.67bcd
Vernonia galamensis	6.67bcd
Vicia faba	20a
Parthenium hysterophorus (Control)	0d
CV (%)	6

Means followed by the same letter(s) within column are not significantly different from each other at ($P \leq 0.05$).

G. scabra, *Flaveria trinervia*, *Tagetes minuta* were screened at flowering stage for their susceptibility to the weevil. These test plants and parthenium were grown in plastic pots (20 × 18 cm) with soil composition of 25% sand and humus each and 50% soil in the nursery. Ten adults (5♂ and 5♀) were released in caged test plants. Parameters of host-specificity test were recorded on setting of insects on plants, oviposition, feeding symptoms and mortality of adult weevils were observed after seven days of release.

Similar sized plants in height (70 cm), number of branches (40–41.33), number of leaves (90.33–93.33) and number of flowers (284–290.33) were selected to assess the optimum number of larvae to kill the parthenium plant. The same experimental procedures were used as host specificity test. Five mated male and female weevils were released on caged parthenium plant for oviposition and weevils were removed from the cage after laying sufficient number of eggs as dose requirement. Dhileepan (2003) estimated that a minimum of five larvae per plant is necessary to prevent the plant from flowering. So, 2, 4, 6, 8, 10 and 12 larvae were released per plant but significant result was not recorded. To decide the effective number of larvae to kill

TABLE 2. Effect of *L. setosipennis* on parthenium after 7 days of larval releases (12 days from egg laying)

No. of larvae per plant	Plant height (cm)	% reduction of plant height over control	Wilted branches (%)	Wilted leaves (%)	Wilted flowers (%)
9.33	71.5b	7.5	0 (3.33)f	34.97 (36.25)f	19.51 (26.21)f
14.33	70.67bc	8.58	15.50 (23.12)e	43.13 (41.05)e	31.68 (34.25)e
20	70.33c	9.02	38.12 (38.10)d	58.33 (49.79)d	46.40 (42.93)d
25.33	70.17c	9.22	60.42 (51.02)c	70.1 (56.87)c	63.12 (52.61)c
29.33	70.17c	9.22	79.14 (62.48)b	90.69 (72.36)b	84.38 (66.75)b
35	70c	9.44	100 (90)a	100 (90)a	100 (90)a
Control (0)	77.3a		0 (3.33)f	2.54 (9.11)g	0.19 (1.16)g
CV (%)	0.95		4.49	2.74	2.98

Means followed by the same letter(s) within column are not significantly different from each other at ($P \leq 0.05$).
Data in parenthesis are Arc Sign transformed value.

the plant, eggs i.e. 10 + 1, 15 + 2, 20 + 3, 25 + 4, 30 + 5 and 35 + 6 were left on parthenium flower heads for hatching to get the tentative number of 10, 15, 20, 25, 30 and 35 larvae, respectively. Actual numbers of larvae were determined after counting the larvae from the stem or pupa in the soil and it was recorded 9.33, 14.33, 20, 25.33, 29.33 and 35 larvae. Control was used to compare the treatments. Observations were made on plant height, number of wilted leaves, number of wilted flowers and number of wilted branches after 12, 19 and 30 days of egg laying (or 7, 14 and 25 days of larval releases because 5 days oviposition period was recorded in another set of experiment) and ultimately total plant biomass, number of roots and root length was observed.

To study the regeneration of parthenium plants damaged by the weevils an experiment with seven treatments viz. 9.67, 15, 21, 26.33, 30.67 and 35 and control were set up. The numbers of larvae were confirmed as shown in the experiment of optimum number of larvae to kill the parthenium. Wilted Plants were left for regeneration after 25 days of larval releases and observations were made on number of newly emerged leaves, branches and flowers and increment on plant height after 7 and 15 days of wilting. These experiments were set up in completely randomized

TABLE 3. Effect of *L. setosipennis* on parthenium after 14 days of larval releases (19 days from egg laying)

No. of larvae per plant	Plant height (cm)	% reduction of plant height over control	Wilted branches (%)	Wilted leaves (%)	Wilted flowers (%)
9.33	72.83b	17.36	26.52 (30.96)e	60.23 (50.91)e	50.22 (45.13)e
14.33	70.67c	19.81	42.52 (40.73)d	73.13 (58.78)d	61.29 (51.53)d
20	70.33c	20.20	69.01 (56.24)c	84.32 (66.76)c	76.56 (61.08)c
25.33	70.17c	20.38	84.69 (66.98)b	93.81 (75.61)b	90.19 (71.78)b
29.33	70.17c	20.33	100 (90)a	100 (90)a	100 (90)a
35	70c	20.57	100 (90)a	100 (90)a	100 (90)a
Control (0)	88.13a		2.44 (8.99)f	4.99 (13.82)f	1.15 (6.08)f
CV (%)	0.95		3.2	2.17	2.15

Means followed by the same letter(s) within column are not significantly different from each other at ($P \leq 0.05$).
Data in parenthesis are Arc Sign transformed value.

design with three replications. Data were analyzed using SAS software and means were separated by Duncan Multiple Range Test. Percent Reduction of plant height, number of roots and root length over control was calculated using the Abbott's formula (1925):

$$\text{Percent Reduction over Control} = \frac{C-T}{C} \times 100$$

Where,

C = Observation in Control

T = Observation in Treated

RESULTS AND DISCUSSION

Among the test plants evaluated feeding symptoms was observed only on parthenium flowers and slightly on leaves under no choice conditions. Female weevils did not settle for egg laying on any tested plants except on parthenium. They were active only on parthenium during night but their activity did not observe on other used test plants at any time and hidden in the soil. The mortality of weevils exposed to the test plants was not observed on *P. hysterophorus* and on *G. scabra* when observed seven

TABLE 4. Effect of *L. setosipennis* on parthenium after 25 days of larval releases (30 days from egg laying)

No. of larvae per plant	Plant height (cm)	% reduction of plant height over control	Wilted branches (%)	Wilted leaves (%)	Wilted flowers (%)
9.33	72.67b	24.87	57.94 (49.59)d	81.78 (64.83)c	76.67 (61.11)e
14.33	70.67c	26.94	81.89 (64.88)c	92.24 (74.05)b	91.84 (73.43)c
20	70.33c	27.29	89.36 (71.17)b	100 (90)a	95.29 (77.54)b
25.33	70.17c	27.46	100 (90)a	100 (90)a	100 (90)a
29.33	70.17c	27.46	100 (90)a	100 (90)a	100 (90)a
35	70c	27.63	100 (90)a	100 (90)a	100 (90)a
Control (0)	96.73a		7.34 (15.57)e	13.17 (21.62)d	1.97 (8.04)e
CV (%)	0.85		3.42	3.03	1.32

Means followed by the same letter(s) within column are not significantly different from each other at ($P \leq 0.05$).
Data in parenthesis are Arc Sign transformed value.

days after release while the percentages on all other used test plants were varied from 3.33 to 20 (Table 1). Wild *et al.* (1992) reported some feeding and development of *L. setosipennis* was only on sun flower and safe bio agent to control parthenium. It was not recorded as a pest of any cultivated crop or pasture in South America (Quintana and Abot, 1987). In the present study, weevils did not encounter damage on used test plants including sunflower except parthenium.

Wilting of branches, leaves and flowers was increased with the advancement of number of larvae. After seven days of larval release, complete wilting of plants was on 35 larvae per plant and >50% wilting of the plants was on 25.33 and 29.33 larvae per plant and plant height was increased significantly on larvae free plant (Table 2). After 14 days of release, complete wilting of plant was recorded on 29.33 and 35 larval populations and >50% wilting of plant was on 25.33, 20 and on 14.33 larvae and 20% plant height was reduced over control on 20 larvae and above on this larval number (Table 3). Data in Table 4 show that hundred per cent wilting of plants was observed on 25.33, 29.33 and 35 larvae per plant, >90% wilting was on 20 larvae, >80% wilting on 14.33 and >50% wilting was on 9.33 larvae after 25 days of larval release. Plant height was reduced >27 % on 20 larvae and above on this larval population after 25

TABLE 5. Effect of *L. setosipennis* on parthenium plant biomass, number of roots and root length after 25 days of larval releases (30 days from egg laying)

No. of larvae per plant	Plant biomass (g)	% reduction of plant biomass over control	No. of roots	% reduction of No. of roots over control	Root length (cm)	% reduction root length over control
9.33	40.73 (11.68)b	41.78	72.67 (15.63)b	21.29	26.8b	7.36
14.33	38.86 (11.34)bc	44.45	65 (14.77)c	29.60	25.9b	10.47
20	36.73 (11.09)cd	47.50	61 (14.29)c	33.93	25b	13.58
25.33	34.3 (10.73)cd	50.97	50 (12.91)d	45.85	22.67c	21.64
29.33	32.26 (10.36)e	53.89	44.66 (12.19)d	51.63	20.17d	30.28
35	25.1 (9.08)f	64.12	32.66 (10.4)e	64.63	15.57e	46.18
Control (0)	69.96 (14.77)a		92.33 (17.69)a		28.93a	
CV (%)	2.78		3.19		4.92	

Means followed by the same letter(s) within column are not significantly different from each other at ($P \leq 0.05$).
Data in parenthesis are Arc Sign transformed value.

days of larval release. Dhileepan (2003) reported that *L. setosipennis* reduced the plant height by 51%, number of leaves by 78%, flower production by 63% in rosette stage of the plants in glasshouse. In the present investigation, the weevil reduced 25% plant height over control and 90% wilting of branches, leaves and flowers were observed on 20 larvae per parthenium plant and complete wilting of plants was on 25.33 larvae and above on this number.

More than 50% plant biomass was reduced over control on 25.33 larvae and above on this larval number. Maximum (64.63%) reduction of number of roots over control was on 35 larvae and minimum (21.29%) on 9.33 larvae and it was found significantly superior to control. Root length was reduced also maximum (46.18%) on 35 larvae and minimum (7.36%) on 9.33 larvae over control (Table 5). Dhileepan (2003) reported that the weevil reduced the plant biomass by 54% in rosette stage of the plants in glasshouse but the impact of it, on total plant height, basal stem width, root length, number of branches and root biomass was not significant in both glasshouse and field cages. In the present study, the weevil reduced plant biomass, number of roots and

root length significantly over control under quarantine conditions due to higher larval populations.

Regeneration capacity of wilted parthenium plants damaged by *L. setosipennis* indicated that no growth in leaves, branches, flowers and increment in plant height was observed on higher larval number i.e. 21, 25.33, 30.67 and 35.67 larvae per plant. However, plant did not wilt completely on lower larval number, therefore, new leaves i.e. 4.67 and 3 were emerged on 9.67 and 15, larvae, respectively, whereas growth in branches, flower and increment in plant height was not observed up to 15 days of observation.

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SEM descriptions of an aquatic thrips, *Organothrips indicus* Bhatti (Thysanoptera: Thripidae)

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ABSTRACT: *Organothrips indicus* Bhatti which is known to live and multiply in submerged condition has been collected from the aquatic plant, *Monochoria vaginalis* and *Eichornia crassipes* (Pontederiaceae). The thrips has been recorded for the first time from the Indo-Myanmar hotspot region of North Eastern India. The descriptions of *O. indicus* using SEM have been provided in this paper along with the key to identify other known species of *Organothrips*. © 2011 Association for Advancement of Entomology

KEYWORDS: aquatic thrips, *Organothrips indicus*, scanning electron microscope, SEM study, NE India

INTRODUCTION

Members of the genus *Organothrips* are unique and very distinctive slender bodied individuals of the subfamily Thripinae. However, they are unusual within the family Thripidae for their gross structure such as strong head projection, forewings strongly recurving apically, legs slender bearing an oar shaped or fimbriate setae on the inner side on each fore-tibia. Individuals of this genus have the following characters. Body clearly bi-colourous, head with prominent anteocular process, maxillary palps 2-segmented, Antennae 8 segmented with simple sense cones on segments 3 & 4, and segment 2 lacking dorsal seta basad of campaniform sensillum, basantra membranous with a pair of setae, ferna divided, all tarsi 1-segmented, median pair metascutal setae in posterior half, meso and metathoracic sterna apophyses widely separate, wing fringes strongly undulated, veinal setae sparsely set, an unusually porous body surface chitin and much smaller abdominal spiracles (Bhatti, 1998; Mound, 2000). The world check list (Mound, 2005—updated as of March, 2012) revealed the record of three species under the genus *Organothrips*, such as *O. bianchii* Hood, 1940 from Hawaii, *O. indicus* Bhatti, 1974 from India and a third member being described from Australia *O.wrighti* Mound, 2000. Of which the former two species are aquatic i.e., their life

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stages occur below water level and the latter one is terrestrial. In the present study, a brief description of the morphological features of *Organothrips indicus* is presented for the first time based on EM study along with the key to identify different species of *Organothrips* so far known. Occurrence of this species in India is known only from Delhi and Darjeeling areas and this is the first report indicating their distribution in biodiversity rich north eastern sectors of India.

MATERIALS AND METHODS

Sixty seven females were collected from Manipur University Campus, Canchipur (Lat 24° 45′ 02.93″ N; Long 93° 55′ 49.23″ E Lattitude 733.5 m ASL) from *Monochoria vaginalis* [new host record] and *Eichornia crassipes* (Pontederiaceae) (Water temperature 26–29°C). Thrips were observed on the slimy milieu at the nodal region and also on the bulbous petioles and buds that are found submerged in the shallow water. Collected specimens were active in water when they were kept in an eppendorf plastic vial for at least 4 hours. These samples were fixed in glutaraldehyde at 4°C for 2 hrs and then transferred to 0.1 M Sodium cacodylate buffer at 25°C for 4 days. Later dehydrated in ethanol series for 10 min each. After drying them at a critical point, the specimens were mounted on the stub using a double side adhesive tap. Subsequently, it is placed inside the sputter gold coat for gold coating (10 + 6 min). The gold coated specimens were once again dried in the desiccator and loaded inside the SEM and viewed on the monitor. The sample was scanned and desired images were captured, under 15 kV emissions current. Later, photos were taken with the table top model of SEM -JEOL JSM 6360. *(`http://semlab.uaa.alaska.edu/protocol.htm`, `http://bama.ua.edu/~emlab/instructions/sem_fix.html`.)

RESULTS AND DISCUSSION

Organothrips indicus Bhatti

Although Bhatti (1974) has made a detailed observation of *O. indicus* using the light microscope, the present observation has shown not much deviation with the exception of additional information on the presence of teeth on the transverse anastomosing lines present on the abdominal terga I to VIII (Fig. 1D & E). The fimbriate setae/pedal like spur at the apex fore-tibia (Fig. 1F) measuring about 15 μm, along with the forwardly curved apical region of forewing appear to help thrips in swimming. It is significant to note here that the apically re-curved forewing is one of the unique features of *Organothrips* (Fig. 1G). Further, presence of complete comb of microtrichia along the posterior margin of terga II to VIII is very much evident in the SEM photos of the specimens examined in the present work. Although Bhatti (1974) has also observed this feature, the key that has been presented highlights the presence of microtrichia only from terga II to V (Bhatti, 1998). This difference in observation is made clear in the present study with the evidence of SEM micrograph (Fig. 1D, E & H). Therefore, a revised key is given here for the members of *Organothrips* based on the descriptive accounts given by the respective authors, which in turn enable us to distinguish the

TABLE 1. Key to species of the genus *Organothrips* (female)

1. Antennae long and slender; body bicolourous in female; fore tibiambriate; fore wing slender	 2
– Antennae short and robust; body unicolourous; fore tibia bid; fore wing broad at apex.	..... *wrighti*Mound, 2000
2. Medial tergite with complete comb on posterior margin on tergite II–VIII. Meso and metasternum subreticulate in middle. Median pair of setae—S1 on abdominal sterna II to VI reduced	..... *indicus* Bhatti, 1974
– Medial tergite comb of microtrichia on posterior margin of tergite II–V lacking in middle. Meso and metasternum unsculptured. Median pair of setae—S1 on abdominal sterna II to VI normal, not shorter than S2	..... *bianchii*Hood, 1940

three species so far known from different parts of the world (Table 1). In addition to it, the following account throws light on the general body colour, chaetotaxy and body measurements to enable to identify this aquatic species easily.

Female macroptera

Body slender, bicoloured; Head, thorax and abdominal segment 1, 2, 3, 6, 7, 8 dark brown, Segments 4, 5, 9, 10 yellow, antennae segment 2 darker than segment 3 but paler than 1, femora uniformly brown except a small pale area at the base of mid femora, all tibia light brown to yellow, wings with 2 cross bands (Fig. 1A, B & I), Posterior margin of abdominal tergites 2–8 with complete comb plates, segment 1 with an incomplete plate in rows of three, two at sides and one in the middle, combs on sterna posterior margin of abdominal segments incomplete lacking at the middle. Total body length 1542, Antennal length (Width) A_1 24(36), A_2 38(23.7), A_3 60(18), A_4 54(18), A_5 45(18), A_6 63(16.8), A_7 22(9), A_8 22(6). Head length 171-109, width across the eye 152-120, across Cheeks 141-105 and at base 126-93, Ocellar setae II 18-12, Distance between I and II ocelli 43-12 and between the basal ocelli (II & III) 59-27, Head projection length (Width) 43-31(108-68), Pronotum length (W) 165-123(213-164), Post angular inner setae 54-37, Post angular outer setae 33-19, length of fore tibial oar shaped setae 18-14, fore wing length 724-514, width at the middle 39-22, Abdomen VII length (W) 81-69(233-192), Abdomen VIII 82-68(197-164), Abdomen IX 99-78(159-124), Abdomen X 102-75(87-63), Ovipositor length 225-177. Wing chaetotaxy: lower vein with only 5 setae, upper vein with 4 2 setae and only 6 costal setae. (All measurements in microns; data based on 13 replications).

Aquatic adaptations of Organothrips

As early as 1969, Titschack discovered that *Organothrips* could complete its life cycle under the surface of water on fully submerged *Cryptocoryne*, even without coming

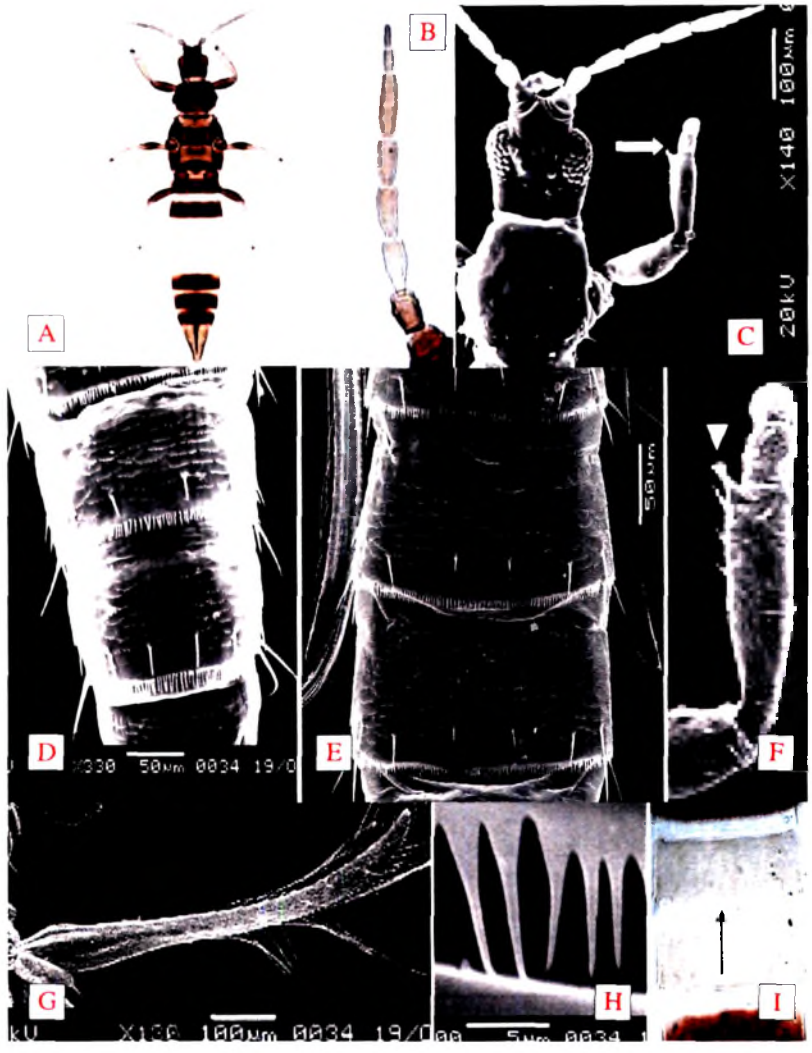


FIGURE 1. *Organothrips indicus*: A–Adult ( ); B—Antenna; C—SEM view of Head, Prothorax and Foreleg; D and E: Tergite: 4–5 & 7–8 showing transverse anastomosing line with teeth and complete set of posteromarginal comb–microtrichia). F—Fimbriate Fore-tibial spur; G—Apically recurved fore wing; H-Enlarged view of microtrichia on tergite; I—Sternite 4 & 5 showing weak sternal microtrichia (Photos A, B, and I have been taken with light microscope (Olympus CX31 & Olympus SLR-E520) and the rest being SEM photos).

to the surface for uptake of oxygen. He was of the opinion that the aquatic thrips could absorb oxygen through the integument having extensive bundles of very ne tracheal branches present directly under the sternal integument. Moritz (1985) had also indicated the possibility of gaseous exchange through exoskeleton into the tracheae

rather than spiracles; since the latter seems to be non functional. Further, the body surface chitin of *O. indicus* has also been found to be unusually porous under high power phase contrast (Mound, 2000). The pedal like spur at the apex of fore tibia enable them to swim in water (Bhatti, 1998) along with the apically re-curved forewing which perhaps act as a rudder on either side. Therefore, *O. indicus* seems to be at ease inside water.

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Gamma radiation sensitivity on larvae and eggs of common mormon butterfly, *P. polytes*

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ABSTRACT: Radiosensitivity of *Papilio polytes* (Lepidoptera) was studied by analyzing the frequency of egg hatchability, larval mortality and fecundity. Gamma radiation treatment to different life forms of *P. polytes* at a dose rate of 5 Gy/min with 8 different dosages (10, 20, 30, 40, 50, 60, 70, and 80 Gy) enabled to determine the LD_{50} values of different life stages of *P. polytes*. In the 18 hr-old 5th instar larva LD_{50} dosage was 56.10 Gy. Mortality rate in the larval forms increased from 9.17 ± 0.83 at 10 Gy to 94.17 ± 0.83 at 80 Gy. Fecundity (number of eggs laid) was found to decrease from 64.33 ± 0.19 at 10 Gy to 1.78 ± 1.78 at 80 Gy. Egg hatchability rate also showed significant decrease from 30 Gy onwards (45.11 ± 0.67) to 80 Gy (0.67 ± 0.67). Larval emergence from non-irradiated normal eggs was 96.67 ± 1.67 that was found to reduce 10.83 ± 2.20 at the dose of 80. A gradual decrease in hatchability was noticed as the radiation dosage increased from 10 Gy to 80 Gy when compared to control groups. Computation of the comprehensive data collected show that increasing radiation dosages elicited significant reduction on survivability, fecundity in different life forms of *P. polytes*. With increasing dosage of irradiation from 10 to 80 Gy gamma radiation frequency of adult emergence from late instar larval forms also showed significant reduction. © 2011 Association for Advancement of Entomology

INTRODUCTION

The frequent occurrence of radionuclide disasters across the world necessitate in depth information on the biological impact and the degree of risk involved on ionizing radiation both the flora and fauna including the humans. However, in the lower groups of animal forms, detailed information on this is significantly lacking. Insects being a diverse group of organisms with their significant role on human welfares undisputable. In adaptive diversity and species number they are the most successful organisms. Insects can be found in almost every conceivable habitat and as a group displays diversity in form and function nearly unapproachable by other animal groups. The unique phenomenon of metamorphosis could be the most important and widespread

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of all these adaptations. Insects not only contribute to more than two-thirds of the biodiversity on earth but also found to play an imperative role both on the economy and health by functioning as pollinating agents, pests and vectors of several infectious diseases. Several of the insect hormones and neuropeptides have also been projected as biopesticidal agents as well (Muraleedharan and Gayathri Elayidam, 2008). Hence insect neuropeptides are promising candidate for high-efficiency cell transfection and nucleus-specific cell labeling.

Insect response to ionizing radiation are often designated to be dependent both on the dosage applied and the specific life stage. Undifferentiated, mitotically active tissues are most sensitive to ionizing radiation. Consequently, eggs are normally the most susceptible life stage while adult forms are the most tolerant life stages. Because insect gonads and midgut, both house mitotically active tissues which are highly susceptible to radiation damage, irradiated insects are often sterile and stop feeding soon after the treatment. Irradiated larvae might delay the development, and may not die until they approach their subsequent molt, resulting in prolongation of its survival time. Extensive investigations have been conducted on the use of radiation to control the stored grain insect pest species (Boshra and Mikhaiel, 2006).

The Common Mormon (*Papilio polytes*) is a common species of swallowtail butterfly widely distributed across Asia. This butterfly is known for the mimicry displayed by the numerous forms of its females which mimic inedible Red-bodied Swallowtails, such as the Common Rose and the Crimson Rose. *P. polytes* belongs to the family Papilionidae. This butterfly is palatable to insectivorous birds. There are about 60 species of *Papilio* in the Oriental region, and 210 species worldwide. The larval food plants include *Citrus, Clausena, Fortunella, Glycosmis, Zanthoxylum, Toddalia, Aegle, Murraya, Triphasia, Euodia, Atalantia and Poncirus (Rutaceae).* This species typically has five distint larval instars. The average life span of the species extends from 26 to 59 days.

The aim of the present investigations were to understand the specific effect of gamma radiation on mortality, fecundity and hatchability of the *P. polytes* insect species. An attempt has also been made to assess the gamma radiation impact on their eggs as well.

The Model Organisms

Insect used for present studies is different life forms of Common Mormon butterfly, *Papilio polytes* (Lepidoptera : Papilionidae),which is a major pest of citrus plants.

Gamma Irradiation Treatment

Irradiation treatments was performed by using Cobalt 60 source from unit Gamma Chamber 5000 (BRIT); at the Meat Technology Unit, Kerala Agriculture University, Mannuthy, Thrissur. Irradiation was carried out at a dose rate of 5 Gy/min at 8 different dosages (10, 20, 30, 40, 50, 60, 70, and 80 Gy). Forty numbers of organisms each were irradiated in three different batches (3 × 40 organisms) for each dosage. Single batch of larvae corresponded to a repetition and each repetition was treated separately. Three

batches with 40 eggs each (3 × 40 eggs) were also irradiated with 10 to 80 Gy gamma rays. Both larvae and eggs of the control group were just transferred to the irradiator and subsequently removed without exposure to any radiation. All such irradiated organisms were monitored to assess their Mortality, Fecundity and Hatchability rate.

Larval Irradiation

P. polytes 5th instar larvae were selected for irradiation studies. All the larvae were irradiated in the same irradiator at varying dose ranges from 10–80 Gy. Immediately after treatment, all irradiated and control larvae were simultaneously transferred to the appropriate culture basins with feed and maintained at the rearing conditions in the respective culture basins. Mortality and adult emergence of these larvae were recorded every 6 hrs interval. Adults that developed from irradiated larvae were removed and their life cycles were recorded. Three replicates (40 larvae/nymphs for each replicate) were used for each dosage with their control.

Eggs Irradiation

Freshly laid eggs (0 day old) of *P. polytes* was collected. Forty eggs were placed in a single glass Petri dish and irradiated in the Gamma Chamber. Radiation dosages applied varied from 10 to 80 Gy to each bach of eggs. Control egg groups were just transferred to the irradiator without any radiation exposure. After irradiation eggs were transferred to the rearing Petri dishes with fresh leaves feed and maintained at 26–30°C. Number of eggs hatched on each day were recorded accurately. Triplicate egg batches were used for each dosage level and their respective control.

Statistical Analysis

All the data collected from were subjected to statistical analysis using appropriate statistical techniques. Statistical tools used varied for different experimental data analysis. Descriptive statistics such as arithmetic mean (AM) and standard error (SE) were calculated for summarizing the collected raw data. For the LD_{50} calculation, Probit Analysis was used. Probability units (Probit) analysis was evaluated by constructing a dose-response regression model (Finney, 1952). One-way analysis of variance (ANOVA) was carried out for finding significant concentration effect with respect for each of study variable (after making a square root transformation for achieving normality assumption) such as mortality, fecundity and hatchability. Two-way analysis of variance (ANOVA) was used for finding significant main effect due to concentration and time and also the interaction effect of concentration and time on respective study variables (after making a square root transformation for achieving normality assumption). The level of significance of the present study was fixed at 5% limit. A calculated P value less than 0.05 is considered to be statistically significant.

RESULTS

LD_{50} for *P. polytes* larvae

LD_{50} calculation for *P. polytes* larvae was done by evaluating the mortality rate of 18 hours in a range of 10 Gy to 80 Gy gamma radiation dose. In order to predict the probit (p), a linear regression model is fitted, the details are given in Table 1. As is evident from this table to get a 25% response, the corresponding dose would be 40.39 Gy, with confidence interval ranging from 36.88 Gy to 43.48 Gy. Likewise for getting a 50% response, the corresponding dose would be 56.10 Gy, with confidence interval ranging from 53.12 Gy to 59.36 Gy. To get 99% mortality for *P. polytes* 5th instar larvae, the gamma radiation dose applied would be 110.28 Gy (102.03 Gy–121.23 Gy).

Gamma Radiation Impact on Mortality

In order to find out the concentration effect with time on mortality, two-way ANOVA was carried out. Since the data distribution do not obey Gaussian law, square root transformation has been done. There was found to exist a significant effect due to concentration ($P < 0.0001$) and time ($P < 0.0001$) on mortality (Table 2). Moreover there was a significant interaction effect due to concentration and time on mortality ($P < 0.0001$) as well. Since the interaction effect is significant, the effect due to concentration (ignoring time) and effect due to time (ignoring concentration) cannot be considered. Highest average mortality was reported at 80 Gy dose during different time periods (Fig. 1).

It was found that there existed a significant increase in the average mortality rate with increase of dosage. As the gamma radiation dose increased from 10 Gy to 80 Gy, the mortality rate also showed a concomitant increase from 9.17 ($\pm$ 0.83) to 94.17 ($\pm$ 0.83) (Table 2). When compared to control (5.83 $\pm$ 0.83), 10 Gy dosage showed little effect on mortality (9.17$\pm$ 0.83). From 20 Gy to 80 Gy, there was a linear increase on mortality rate. Considering the effect of time on mortality, it was evident that as time increased with the radiation dosage, average mortality rate also showed a corresponding increasing trend (Fig. 1).

Gamma Radiation Impact on Fecundity

The adults survived after irradiation when reared further for monitoring fecundity rate. At each irradiated dosage when three different survived pairs were monitored showed significant variation in fecundity rate ($P < 0.0001$) with increasing dosage (Table 3). The overall impact of varying dosages of irradiation on fecundity on treated and survived moths on Fecundity is depicted in Fig. 2. Scheffe's test further showed that there do not exist any fecundity difference between concentrations at 0 Gy (66.33 $\pm$ 0.19), 10 Gy (64.33 $\pm$ 0.19) or at 20 Gy (61.56 $\pm$ 0.59). The average fecundity rate was found to be inversely proportional as the gamma radiation dosage steadily increased from 30 Gy (55.00 $\pm$ 0.88) to 80 Gy (1.78 $\pm$ 1.78). Overall data analysis show that the fecundity rate of the organism is affected only with higher gamma radiation dosages.

TABLE 1. Probit Analysis Values of Mortality Rate of *P. polytes* larvae (18 hrs)

Probit (%)	18 hour			Probit (%)	18 hour		
	Dose	LCL	UCL		Dose	LCL	UCL
0.01	1.92	—	8.68	0.55	59.03	55.95	62.51
0.02	8.27	0.39	14.29	0.60	62.00	58.78	65.77
0.03	12.29	5.05	17.86	0.65	65.07	61.65	69.17
0.04	15.32	8.55	20.56	0.70	68.31	64.65	72.80
0.05	17.79	11.39	22.76	0.75	71.81	67.84	76.75
0.06	19.89	13.80	24.64	0.80	75.70	71.37	81.19
0.07	21.73	15.91	26.29	0.85	80.24	75.45	86.39
0.08	23.37	17.80	27.77	0.90	85.95	80.54	92.96
0.09	24.87	19.51	29.12	0.91	87.33	81.77	94.56
0.10	26.25	21.08	30.37	0.92	88.82	83.10	96.29
0.15	31.96	27.54	35.58	0.93	90.47	84.56	98.20
0.20	36.50	32.60	39.79	0.94	92.31	86.19	100.33
0.25	40.39	36.88	43.48	0.95	94.41	88.04	102.76
0.30	43.89	40.64	46.86	0.96	96.87	90.22	105.62
0.35	47.12	44.06	50.07	0.97	99.90	92.90	109.15
0.40	50.20	47.23	53.18	0.98	103.93	96.45	113.83
0.45	53.17	50.23	56.26	0.99	110.28	102.03	121.23
0.50	**56.10**	**53.12**	**59.36**				

TABLE 2. Test of Signicance of Gamma Radiation Effect –Radiation Dosage VS Time on Mortality Rate of *P. polytes*

Dose (Gy)	18 hour		24 hour		30 hour		Total	
	AM	SE	AM	SE	AM	SE	AM	SE
0	0.00	0.00	0.00	0.00	5.83	0.83	1.94	1.00
10	0.00	0.00	0.00	0.00	9.17	0.83	3.06	1.55
20	11.67	0.83	25.83	0.83	35.83	0.83	24.44	3.53
30	15.83	1.67	33.33	1.67	45.00	1.44	31.39	4.31
40	30.00	0.00	51.67	0.83	58.33	0.83	46.67	4.29
50	36.67	0.83	45.00	0.00	61.67	0.83	47.78	3.69
60	40.00	0.00	54.17	0.83	75.83	0.83	56.67	5.22
70	72.50	0.00	74.17	0.83	80.00	0.00	75.56	1.16
80	94.17	0.83	94.17	0.83	94.17	0.83	94.17	0.42
Total	33.43	5.96	42.04	5.80	51.76	5.68	42.41	3.42

$P_{Concentration}$ 0 0001 , P_{Time} 0 0001 , $P_{Concentration\ Time}$ 0 0001

Gamma Radiation Effect on Hatchability

The eggs laid by the irradiated (10 Gy to 80 Gy) *P. polytes* adults were incubated for hatching. Hatchability rate was noted every day until there were no further hatch

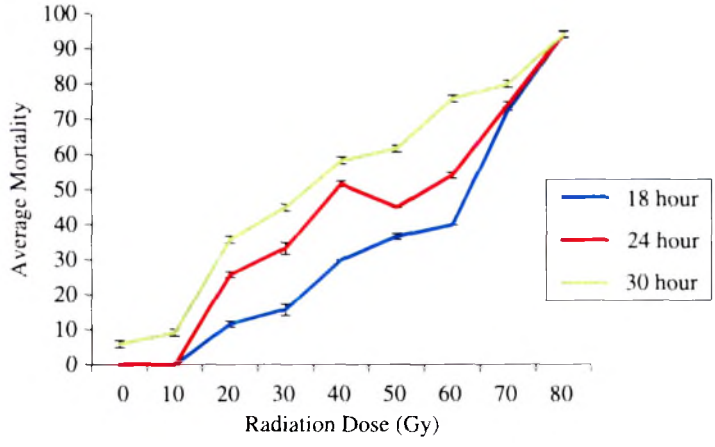


FIGURE 1. Graph showing gamma radiation effect on mortality rate of *P. polytes*

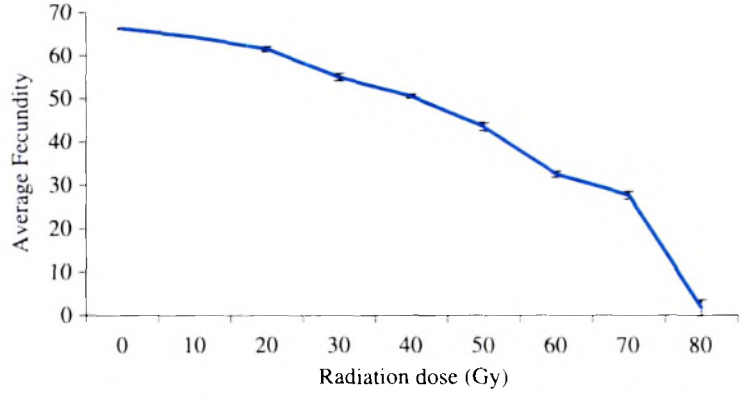


FIGURE 2. Graph showing gamma radiation effect on Fecundity Rate of *P. polytes*

outs and it was observed that on 4th and 5th days after egg laying and the data is as represented in Table 4. There was a signicant difference in hatchability (P 0.0001) at different gamma radiation dosages.

No hatchability difference between 10 Gy (55.22 0.29) and 20 Gy (54.78 0.22) gamma radiation dosages compared to the control (59.00 0.39). When the data analyzed using Scheffe's test from 30 Gy to 80 Gy, the average hatchability rate showed a decrease from 45.11 0.67 to 0.67 0.67. The results are summarized as in Fig. 3. From the data it may be inferred that even though the irradiated adults lay eggs, their hatchability rate is signicantly decreased. Showing that gamma radiation have a profound effect both on fecundity and hatchability of the organism. Freshly laid (0 day old) eggs of *P. polytes* when irradiated signicant reduction (P 0.0001) in egg

TABLE 3. Showing Gamma Radiation Effect on Fecundity rate of *P. polytes*

Dose (Gy)	Fecundity AM	SE
0	66.33	0.19[a]
10	64.33	0.19[a]
20	61.56	0.59[a]
30	55.00	0.88[b]
40	50.56	0.48[c]
50	43.45	0.97[d]
60	32.56	0.68[e]
70	27.67	0.88[f]
80	1.78	1.78[g]
P	0 0001	

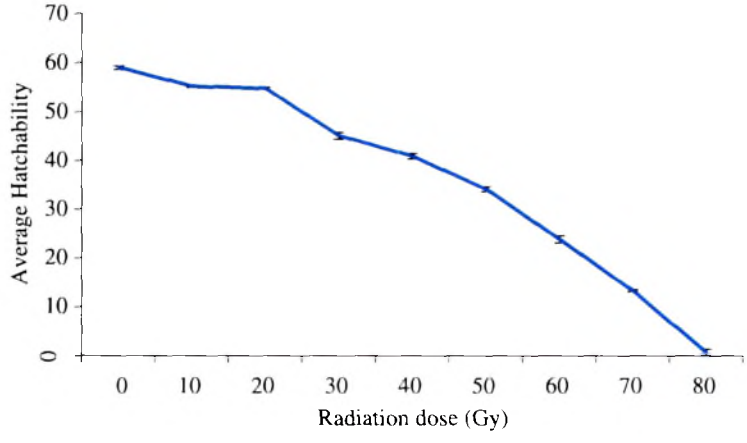


FIGURE 3. Graph showing the of gamma radiation effect on average hatchability rate of *P. polytes*

hatch was observed that was correlated with the radiation dosages to see the effect of gamma radiation on the number of eggs. The data obtained from experiments dealing with irradiation of eggs are summarized in Table 5.

Average larval emergence from irradiated eggs was 96.67 1.67 in the untreated control but was reduced to 10.83 2.20 at the dose of 80 Gy. There was a gradual decrease in hatchability as the radiation dose increased from 10 Gy to 80 Gy when compared to control (Fig. 4). The emerged larvae that survived to the adult stage were also decreased with increasing radiation doses. Statistical analysis of data indicated that irradiation signicantly affected hatchability rate of irradiated eggs.

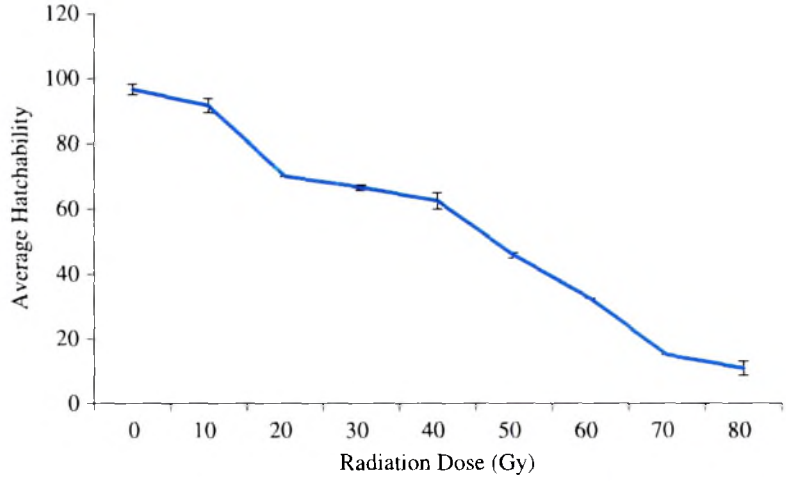


FIGURE 4. Graph showing the effect of gamma radiation effect on average hatchability rate of *P. polytes* eggs

TABLE 4. Showing the Gamma Radiation Effect on Hatchability Rate of *P. polytes*

Dose (Gy)	Hatchability	
	AM	SE
0	59.00	0.39^{b}
10	55.22	0.29^{a}
20	54.78	0.22^{a}
30	45.11	0.67^{c}
40	40.89	0.56^{d}
50	34.11	0.49^{e}
60	23.78	0.73^{f}
70	13.22	0.22^{g}
80	0.67	0.67^{h}
P	0 0001	

DISCUSSION

Monitoring the overall gamma radiation impact assessment and sensitivity on the different life stages of the lepidopteran moth *P. polytes* at varying dosages specically on the mortality rate, egg hatchability and fecundity bring to light many signicant points. In general increasing radiation dosages generally tend to reduce survivability, rate of adult emergence, and the number of eggs produced when fth instar larvae were irradiated with an increasing dosages from 10 to 80 Gy at the rate of 5 Gy/min. Calculated LD_{50} values of *P. polytes* 5th instar larvae after 18 hrs of treatment was

TABLE 5. Showing the Gamma Radiation Effect on Hatchability rate of *P. polytes* eggs

Dose (Gy)	Hatchability
	AM ± SE
0	96.67 ± 1.67^{a}
10	91.67 ± 2.20^{b}
20	70.00 ± 0.00^{c}
30	66.67 ± 0.83^{d}
40	62.50 ± 2.50^{e}
50	45.83 ± 0.83^{f}
60	32.50 ± 0.00^{g}
70	15.00 ± 0.00^{h}
80	10.83 ± 2.20^{i}
	$P = 0.0001^{**}$

56.10 Gy since our present findings showed that in order to get 99% mortality specific irradiation dosage used was 110.28 Gy.

It is generally acknowledged that dose rate has a significant impact on the LD_{50} value in mammals (Chee *et al.*, 1979). In several insect species such an abnormally increased rate of LD_{50} values of radiation dosages have been noted as in female *Sitotroga cerealella* (190.1 K rad) while in males forms (183.200 K rad) for males, for pupae (6.039 K rad), for 3 day-old eggs (5.57 K rad), in 7-day-old larvae (4.800 K rad), in 1-day-old eggs (4.181 K rad) and larvae after 15 days, the noted LD_{50} value was 3.056 Krad (Ismail *et al.*, 1987). The LD_{50} value noted in *A. proylei eggs* was 13 Gy while that in *B. mori it was only* 8 Gy (Akita *et al.*, 1965). The LD_{50} of gamma radiation from cobalt-60 against the body louse (*Pediculus humanus humanus* L.), house fly (*Musca domestica* L.), American cockroach (*Periplaneta americana* (L.)), German cockroach (*Blattella germanica* (L.)), firebrat (*Thermobia domestica* (Pack.)), bed bug (*Cimex lectularius* L.), and Pharaoh ant (*Monomorium pharaonis* (L.)) ranged from 130 r for half- day-old house fly eggs to 190,000 r for body louse nymphs and Pharaoh ant queens. Doses required to cause 100% mortality ranged from 600 r to 250,000 r. Among the species tested, the LD_{50} varied inversely with the size of the insect (Cole *et al.*, 1959), in mammals (Chee *et al.*, 1979). The LD_{50} of the different stages of *Sitotroga cerealella* (Olivier) to gamma radiation was found to be adults (190.100 K rad) for females and 183.200 K rad for males, pupae (6.039 K rad), 3 days old eggs (5.570 K rad), one week old larvae (4.800 K rad), 1-day-old eggs (4.181 K rad) and 2 weeks old larvae (3.056 K rad) (Ismail *et al.*, 1987). The LD_{50} value of 12 hrs old eggs of *A. proylei* found to be around 13 Gy. The LD_{50} for *B. mori* eggs, a few hr after oviposition, was 8 Gy (Akita *et al.*, 1965). In the body louse *Pediculus humanus humanus*, in the house fly *Musca domestica*, and in American cockroaches like *Periplaneta Americana* and *Blattella germanica*, in firebrat *Thermobia domestica*, bed bug *Cimex lectularius*, and in Pharaoh ant *Monomorium*

pharaonis gamma radiation dosages required to cause 100% mortality showed such a wide variation from 600 r to 250,000 r. Further, Cole *et al.* (1959) noted that among almost all the species tested, the LD_{50} values varied inversely with the size of the insect (Cole *et al.*, 1959). Hence such an enhanced dosage level and LD_{50} value noticed in *P. polytes* in our present study is justifiable. Biomolecular rationale for such an enhanced level of radiation resistance noted in the insect *P. polytes* is again in line with our earlier findings (unpublished data) and also as has been reported by Koval (1980) such that the enhanced insect radiation sensitivity could very well be attributed to such an efficient DNA repair mechanism generally existent in insects compared to other animal groups.

Statistically validated and depicted survival or mortality curves against varying dosages and time intervals logically and quantitatively represent the radiosensitivity of the insect studied organism. When *P. polytes* larvae were irradiated with a dosage of 10 Gy to 80 Gy, the average mortality rate also increased from 9.17 ± 0.83 to 94.17 ± 0.83. Among vertebrates, mammals are generally known to be more radiosensitive than birds, amphibians and reptiles while invertebrates mostly have been shown to be much more resistant than the vertebrates (Bacq and Alexander, 1961). Great variations in radiosensitivity has been noticed among a single species and even in the same individual radiation sensitivity showed significant variation depending on the sex and even age of the individual species. In the same individual organism radiosensitivity variations have been noted even among different cell and tissue (Yarmonenko, 1988).

Dosage-dependent enhancement of mortality rate noted in *P. polytes* during the present study again appears logical as the same trend has already been reported in several other insect species earlier as well. In *Periplaneta americana* the lethal dosage of radiation was reported to be 20 K rads while that in *D. melanogaster* and in the parasitoid wasp *Habrobracon* LD_{100} values were 64 K rads and 180 K rads respectively (Wharton and Wharton, 1959).

Sublethal gamma radiation dosage effect (10–100 Krad) on fecundity, longevity and sterility of adults of *Silotroga cerealella* adults showed that the fecundity rate was inversely proportional to the iraradiation dosage. At 100 Krad no eggs were deposited and while at a dosage of 100 Krads significant shortening of the longevity (Ismail *et al.*, 1987). In the Mediterranean flour moth *Ephestia kuehniella*, the number of adults that developed from irradiated eggs and larvae was significantly lower than the untreated controls. Dosage as 200 Gy and above prevented adult emergence from irradiated eggs and hence 250 Gy dosage was selected to prevent complete adult emergence from last instar larvae. Developmental periods, fecundity and egg hatchability were decreased depending on the dosages applied. No eggs hatched at a dosage of 300 Gy and above (Abdurrahman Ayvaz and Aydýn Þ. Tunçbilek, 2006). Adult emergence in fruit flies was prevented when 60–250 Gy of gamma dose was applied. Several groups of pest insect species such as fruit flies, white flies, weevils and scarab beetles all when given a dosage <100 Gy prevented adult emergence completely while some lepidopteran insect pests and most of the mite species required a dosage about 300 Gy (Halima Sadia Khan and Saiful Islam, 2006). Irradiation

of pharate adults of *Anopheles stephensi* with 100 Gy dosage resulted in reduced emergence rate and longevity at a dosage rate varying from 0–70 Gy (Kavita Yadav *et al.*, 2010).

Significant reduction of hatchability rate and reduction of the larval duration in relation to increased radiation dosage of the irradiated eggs noticed in *P. polytes*. Ducoff (1972) earlier showed that irradiation of the cotton bug, *Dysdercus koenigii* nymphs leads to lethality, delay in pupation, developmental abnormalities, failure of emergence and imaginal death soon after eclosion. Nymphal development, reproductive capacity and mating competitiveness of the female also noticed in irradiated bugs (Jayaraman and Mukherjee, 1980). In the housefly, *Musca domestica* dose dependant pupariation delay and disfigured abnormal pupation at higher dosages were noticed (Srinivasan and Kesavan, 1979). Lepidopteran insects are known to be more radioresistant than other insect groups like Hemiptera, Diptera and Homoptera. Koval (1983) showed that the mitotically active cells are approximately 50–100 times more radioresistant than the mammalian cells Koval (1983). Higher radioresistance attributed to lepidopteran cells have been designated to be as a consequence of their possession of holokinetic chromosome with spread out centromeres (Murakami and Imai, 1974). Present studies further reveal that *P. polytes* larvae are more resistant to radiation than the eggs and there is significant variation in susceptibility between different larval instars as well.

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Mound distribution and management of termites species using botanicals and entomopathogenic fungi in Metekel zone of Ethiopia

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ABSTRACT: The field experiments were conducted during 2010 to estimate mound number per hectare, percent maize infestation level, number of termites per maize plant and evaluate the effectiveness of botanicals and entomopathogenic fungi in maize field in Metekel zone, Ethiopia. Statistical correlation indicated that percent maize infestation level increased significantly ($P < 0.05$) with increment of mounds per hectare ($r = 0.44$) and termite number per plant ($r = 0.38$). However, non-significant negative correlation was recorded between altitude and number of mounds per hectare, % infestation level and termite number per plant. Treatment's effectiveness showed that 20% of vetiver, *Vetiveria zizanioides* L. and birbira, *Millettia ferruginea* (Hochst.) Baker were found effective up to 60 days of treatment's application, however, 10% of its concentration were found effective up to 30 days by using three observations methods i.e. bamboo bait, percent maize infested plants and termite number per plant. *Beauveria bassiana* Balsamo and *Metarhizium anisopliae* Metchnikoff were found less effective but gradually increased with time. Higher concentration of vetiver and birbira is recommended for management of termites in maize crop and future studies are needed to develop commercial formulation of the tested botanicals against termites.

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KEYWORDS: Isoptera, Termites, *Vetiveria zizanioides* L., *Millettia ferruginea* (Hochst.) Baker, entomopathogenic fungi, *Metarhizium anisopliae* Metchnikoff and *Beauveria bassiana* Balsamo

INTRODUCTION

Termites are the primary insect group adapted to consume wood and lignocelluloses. Among the 2700 termite species, about 83 were considered as pests and have severe

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impact in tropical and subtropical regions (Hussenederk *et al.*, 2003). Termites damage about 10–30% of harvested kernels of groundnut in Mali, Burkina-Faso, Niger, and Nigeria (Umeh *et al.*, 1999) 15–25% estimated loss in maize in India (Joshi *et al.*, 2005). In Western Ethiopia, *Macrotermes subhyalinus* was observed to cause up to 30% stand loss in maize (Abdurahman, 1990). Djirata *et al.* (2007) reported that five genera i.e. *Ancistrotermes, Macrotermes, Microtermes, Odontotermes, Pseudacanthotermes* from sub-family Macrotermitinae and one genus i.e. *Trinervitermes* from sub-family Nasutitermitinae of Termitidae family caused damage on *Zea mays* L., *Eragrostis tef* (Zuccagni) Trotter, *Sorghum bicolor* L. and rangelands in western part of Ethiopia and farmers were abandoned their crop fields and moved to the lowland, searching for new land due to severe damage. Among the numerous problems, *Macrotermes* sp. constructed 3–5 large mounds per hectare and control was attempted by poisoning mounds with aldrin, phostoxin and other insecticides, removing queens from mounds and increasing the seed rate (Wood, 1991).

Effectiveness of several botanicals and their products have been tested against termites by many researches for instance neem, *Azadirachta indica* (Grace and Yates, 1992; Singh and Singh, 2002); vetiver, *Vetiveria zizanioides* (Zhu *et al.*, 2001; Maistrello *et al.*, 2001; Henderson *et al.*, 2005; Nix Karen *et al.*, 2006; Su, 2005); birbira, *Millettia ferruginea* (Daniel and Bekele, 2006; Bekele *et al.*, 2002); leaves of *Lantana camara, Parthenium hysterophorus, Tagetes erecta* (Sharma *et al.*, 1999). Effectiveness of vetiver was not tested against termites in Ethiopia under laboratory and field conditions. However, birbira was tested by Daniel and Bekele (2006) and he found termiticidal activity against *Macrotermes* sp. in Ethiopia. So far, no scientific evidence is documented on the effectiveness of botanical against termite's fauna in Metekel Zone of Ethiopia. Extensive studies have been done on effectiveness of entomopathogenic fungi especially *Metarhizium anisopliae* and *Beauveria bassiana* against several species of termites in laboratory and field condition world widely (Hanel, 1982; Milner and Staples, 1995; Gitonga, 1996; Milner *et al.*, 1998; Milner, 2000; Milner *et al.*, 2002) (Krutmuang and Supamit, 2005) and in Ethiopia (Haimanot, 2002; Anonymous, 2009). Some studies have been reported also on defensive mechanism of termites against entomopathogenic fungi by Rosengaus *et al.* (1999); Wright *et al.* (2000); Siderhust *et al.* (2005). Thus, this field study was centered on abundance of mound density and management of termites' species through botanicals in Metekel Zone of Ethiopia.

MATERIALS AND METHODS

The field experiments on management of termites using botanicals and entomopathogenic fungi were conducted at Pawe special woreda (district) and the mound distribution study was carried out in 14 villages of five Woredas (strata) i.e. Pawe, Gublak, Mandura, Dibate and Bullen of Metekel Zone of Benshangul Gumuz Regional State, Pawe, Ethiopia during cropping season of 2010. Stratified systematic sampling was used to estimate the mound distribution, % infestation level and termite number/plant. Observations on mound number per hectare were taken at three points

(sampling units) from each stratum at 10–15 km intervals during September 25–30 when maize crop was in reproductive stage. Simultaneously, the observations on % infestation level were calculated from six sampling units (4 × 4 m) by counting the number of infested plants and total number of plants. Six termite infested maize plants were selected randomly from each sampling unit to count the number of termites/maize plant from the soil up to depth of 20 cm, the root and the stem. Survey data on mound density was used to correlate with the altitude, % infestation level and termite number per plant.

The active mounds were selected from maize field to check the efficacy of treatments where similar management practices and the same local maize variety were used in Pawe district. Its activeness were confirmed by putting dried grass and wooden sticks on the mound to forage by workers and by digging 10–20 cm in depth to see live termites. Two botanicals viz. crude seed extract of 'Birbira' (*Millettia ferrugenia* (Hochst) Baker) and boiled root extract of 'Vetiver' grass (*Vetiveria zizanioides* L.) at 10 and 20% w/v, two entomopathogenic fungi i.e. *Beauveria bassiana* (9609 strain) and *Metarhizium anisopliae* (MM strain) at 10g spores per mound, Standard check (0.10% Chlorpyrifos 48 EC) and untreated (local check) were used to check their effectiveness. The prepared solutions in 5 litres of water sprayed into the mound through main galleries using knapsack sprayer (nozzle was detached from the sprayer) and opened galleries were covered with soil immediately after application to prevent evaporation from direct sunlight and birds to eat poisoned dead termites. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications and each mound was treated as one replication. To avoid the biasness in the selection of mound, small, medium and large sized mounds measured in diameter i.e. 1.02–2.84m, 2.85–3.74m and 3.75–4.29m and in height i.e. 0.63–1.50m, 1.51–2.1m and 2.1–2.67m, respectively. The data was analyzed using SAS version 9.2 statistical software. Percent reduction over control was calculated using the Abbott's Formula (1925):

$$\text{Percent Reduction over Control} = \frac{C - T}{C} \times 100$$

Where,

C = Population in Control

T = Population in Treated

Effectiveness of the treatments was assessed by following three methods:

(1) Application of bamboo baits

Application and installation method of baits was followed as described by Verkerk and Bravery (2001). Bamboo bait was used instead of scot pine, *Pine sylvestris* in the present study and it was easily available and preferred by the termites in the study area. Bamboo stakes were measured 4.5 cm in diameter and 45 cm in length. Four baits were installed at 3 m radius from the mound in North, South, East and West directions

and 22% of the bamboo stake was inserted in the soil. Numbers of termites were counted from baits interiorly because termites forage the bamboo stakes interiorly due to hollowed nature of bamboo and might be due to presence of high cellulosic content and rough surface than the outer surface. Observations were taken 15, 30, 45, 60, 70, and 80 days after treatment's application. The same bait was used after each observation in the same hole until it was not fully consumed.

(2) Percent infestation level

Observations were taken from three sampling units (4 × 4 m) per mound in the standing maize field at the distance of 5–10 meter radius to the mound. Percent maize infestation level is the proportion of infested maize plants over the total maize stands and observations were recorded after 15 and 30 days of treatment's application.

(3) The number of termites

It was counted from six uprooted infested maize plants along with the soil and also from the soil in a depth of 20 cm, the root and the stem of the maize from each mound after 15 and 30 days of treatment's application.

RESULTS AND DISCUSSION

Data in Table 1 show the average number of mounds/ha, % maize infestation level and number of termites/maize plant were varied from 2.67–4.83, 2.61–19.23 and 45.25–117, respectively, in studied districts of Metekel zone. Statistical analysis indicates that altitude was negatively correlated to number of mounds per hectare ($r = -0.09$), % maize infestation level ($r = -0.06$) and termite number/plant ($r = -0.09$) but it was non-significant (Table 2). Significant positive correlation was recorded between percent infestation level with number of mounds/hectare ($r = 0.44$) and termite number/plant ($r = 0.38$). *Macrotermes* constructed 5.5 in cultivated areas and 7.9 mounds/ha in grass lands (Abdurahman, 1990) and 3 to 5 (Wood, 1991) in Ethiopia. *Macrotermes goliath* can build as many as 17 mounds/ha (Pearce, 1997), however, *Amitermes vitiosus* cross >200 mounds (Lepage and Darlington JPEC, 2000). Eggleton *et al.* (1994) stated that generic richness of termite decline with increasing latitude. In the present study, average number of mounds/ha was 3.62 and % infestation level and termite number/plant increase with increment of mounds/ha.

Seven termite genera i.e. *Odontotermes, Microcerotermes, Macrotermes, Microtermes, Coptotermes, Psedocanthotermes* and *Trinervitermes* were identified in another set of experiment to cause damage in root zone, fallen stem and cobs of maize crops in the study area and efficacy of the treatments were recorded on these termites' species. More than 50% reduction of termite population over control is regarded as a threshold level of effectiveness. Table 3 represents the overall performance of the treatments using bamboo bait method that 10% of birbira and vetiver were effective up to only 15 and 45 days after treatment's application, respectively. However, 20% concentrations of both the treatments were found effective up to 60 days after treatment's application.

TABLE 1. Mound density, percent maize infestation level, and number of termites per maize plant in Metekel Zone

Districts	No. of mounds/ha	Per cent Maize Infestation Level	No. of termite per maize Plant
Dangur	2.67	10.08	91.81
Mandura	4.83	2.61	45.25
Pawe	4.33	16.93	108
Bullen	3.25	15.09	76.75
Dibate	3.75	19.20	117
Mean	3.62	14.23	92.41

TABLE 2. Correlation between altitude, mound numbers, percent infestation and termite number in Metekel Zone

	Altitude	Mound number per hectare	% Infestation Level	Termite number per plant
Altitude	1.00	−0.09 (0.65)	−0.06 (0.77)	−0.09 (0.65)
Mound number per hectare	−0.09 (0.65)	1.00	0.44* (0.02)	0.11 (0.60)
% Infestation level	−0.06 (0.77)	0.44* (0.02)	1.00	0.38* (0.05)
Termite number per plant	−0.09 (0.65)	0.11 (0.60)	0.38* (0.05)	1.00

*Indicates that values are significant at ($P < 0.05$).

B. basiana and *M. anisopliae* were not found effective in the present study because percent reduction of termite population over control was below 50% even up to 80 days after treatment's application but its effect was gradually increased. Residue of standard check i.e. chlorpyrifos was persistent up long time i.e. 80 days, therefore, it gave more than 70% reduction of termite population over control.

Table 4 indicates the effectiveness of treatments using per cent infested maize plants that all the treatments except entomopathogenic fungi were effective up to 30 days after treatment's application and gave >50% reduction of termite infested maize plants over control. Performance of both the tested entomopathogenic fungi was gradually increased from 33.86 to 36.42% and 25.06–27.81% in *B. bassiana* and *M. anisopliae* after 15 and 30 days of treatment's application, respectively.

The chemical components i.e. nootkatone, a-cedrene, zizanol and bicyclovetivenol from vetiver were found repellent and toxic to termites (Henderson *et al.*, 2005; Zhu *et al.*, 2001); disrupt behavior and physiology of *Coptotermes formosanus* as a consequence of direct physical contact, ingestion, or exposure to the vapors (Maistrello *et al.*, 2001). Bekele *et al.* (2002) reported that rotenone is one of the dominant compounds found in the seed and stem bark of birbira, *M. ferruginea*.

TABLE 3. Effectiveness of Botanicals and Entomopathogenic fungi against termites using Bamboo bait method

Sl. No	Treatments	Number of termites per Bait and Percent reduction of termite population over control after treatment's application (in days)					
		15		30		45	
		No. of termite	% Redn over control	No. of termite	% Redn over control	No. of termite	% Redn over control
1	Birbira seed 10%	99.92cb (9.98)	54.99	133.75c (11.56)	38.48	125.33c (11.17)	35.40
2	Birbira seed 20%	76.67de (8.75)	65.46	81.42de (8.99)	62.55	86.33d (9.28)	55.50
3	Vetiver Root 10%	109.02c (10.43)	50.89	115.17cd (10.70)	47.03	96.42d (9.80)	50.30
4	Vetiver Root 20%	63.00ef (7.91)	71.62	66.92e (8.15)	69.22	76.42d (8.72)	60.61
5	*Beauveria basiana*	191.00b (13.81)	13.96	172.3b (13.08)	20.75	145.42bc (12.05)	25.04
6	*Metarhizium anisopliae*	212.00ab (14.53)	4.50	194.75ab (13.95)	10.43	169.92ab (13.01)	12.41
7	Chlorpyrifos 48% EC	42.42f (6.75)	80.89	47.08e (6.77)	78.35	48.25e (6.86)	75.13
8	Control	222.00a (14.89)	0.00	217.42a (14.71)	0.00	194.00a (13.90)	0.00
CV		10.67		15.50		13.34	
		60		70		80	
		No. of termites	% Redn over control	No. of termites	% Redn over control	No. of termites	% Redn over control
1	Birbira 10%	112.58cde (10.18)	32.01	104.42bc (10.19)	25.37	70.67ab (8.39)	20.44
2	Birbira 20%	85.33 (9.12)	48.47	88.25c (9.38)	36.93	61.25b (7.75)	31.05
3	Vetiver Root 10%	100.42cde (10.02)	39.35	97.17bc (9.84)	30.55	67.25b (8.17)	24.29
4	Vetiver Root 20%	80.67e (8.88)	51.28	85.33c (9.22)	39.02	59.08b (7.67)	33.49
5	*Beauveria basiana*	105.33cd (10.20)	36.39	87.67c (9.33)	37.34	54.75b (7.39)	38.37
6	*Metarhizium anisopliae*	138.83b (11.74)	16.16	114.92b (10.72)	17.87	67.25b (8.06)	24.29
7	Chlorpyrifos 48% EC	43.13f (6.46)	73.95	38.33d (6.04)	72.61	26.50c (4.99)	70.17
8	Control (local check)	165.58a (12.86)	0.00	139.92a (11.82)	0.00	88.83 (9.42)a	0.00
CV		12.78		11.56		17.44	

Means within a column followed by the same letter are not significantly different ($P < 0.05$) and data given in parenthesis are square root transformed values.

TABLE 4. Effectiveness of botanicals and Entomopathogenic fungi against termites using percent maize infestation level

Sl. No.	Treatments	Per cent Maize infestation Level and Percent reduction of termite infested plants over control after treatment's application (in days)			
		15 days		30 days	
		% Infestation	% Reduction over control	% Infestation	% Reduction over control
1	Birbira Seed 10%	13.55c (21.20)	61.74	16.56abc (23.77)	50.64
2	Birbira Seed 20%	8.44cd (16.70)	76.19	10.44cd (18.63)	68.88
3	Vetiver Root 10%	11.56c (19.47)	67.33	14.56bcd (22.50)	56.60
4	Vetiver Root 20%	7.67cd (16.03)	78.36	8.89cd (17.40)	73.50
5	*Beauveria basiana*	23.44b (28.80)	33.86	21.33abc (25.00)	36.42
6	*Metarhizium anisopliae*	26.56ab (31.00)	25.06	24.22ab (29.63)	27.81
7	Chlorpyrifos 48% EC	6.44d (13.53)	81.83	7.44d (15.20)	77.82
8	Control	35.44a (36.13)	0.00	33.55a 33.55a	0.00
CV		14.78		20.00	

Means within a column followed by the same letter are not significantly different ($P < 0.05$) and data given in parenthesis are angular transformed values.

Daniel and Bekele (2006) found that 25% birbira seed crude extract caused 100% mortality of *Macrotermes spp* in filter paper bioassay, whereas, 10% caused 93% mortality. Present study shows the effectiveness of vetiver root extract up to 60 days was next to chlorpyrifos. Its alkaloids decreased tunneling activity and showed a "lingering behavior". Mounds treated with vetiver were found inactive up to 75 days of treatment's application in another set of experiment. Birbira at higher concentration do have also a comparable effectiveness up to 60 days like vetiver due to the presence of bioactive ingredients.

Twenty per cent of birbira and vetiver and 10% vetiver reduced the termite population more than 50% over control up 30 days, however, birbira 10% was effective up to only 15 days of treatment's application using the method number of termites/maize plant. Effectiveness of *B. basiana* was recorded significantly better than *M. anisopliae* but both of them reduced <25% termite population over control (Table 5).

B. bassiana and *M. anisopliae* were not found effective in the present study and did not reach threshold level of effectiveness up to 80 days of treatment's

TABLE 5. Effectiveness of botanicals and Entomopathogenic fungi against termites using number of termites per plant

Sl. No.	Treatments	Number of termites per maize plant and percent reduction of termite population per plant over control after treatment's application (in days)			
		15		30	
		Termite number per plant	% reduction over control	Termite number per plant	% reduction over control
1	Birbira Seed 10%	65.44c (8.09)	56.21	81.78c (9.04)	46.66
2	Birbira Seed 20%	40.67d (6.38)	72.79	50.56d (7.11)	67.03
3	Vetiver Root 10%	62.57c (7.91)	58.13	76.22c (8.73)	50.29
4	Vetiver Root 20%	36.44d (6.04)	75.62	45.56d (6.75)	70.29
5	*Beauveria basiana*	119.01b (10.91)	20.36	117.55b (10.87)	23.34
6	*Metarhizium anisopliae*	135.00a (11.62)	9.66	131.55b (11.74)	14.20
7	Chlorpyrifos 48% EC	25.33d (5.03)	83.05	38.22d (6.18)	75.07
8	Control	149.44a (12.22)	0.00	(12.78) (12.78)	0.00
CV		11.37		10.72	

Means within a column followed by the same letter are not significantly different ($P < 0.05$) and data given in parenthesis are square root transformed values.

application but its effect was increased gradually with the advancement of the days and this finding is agreed with Anonymous (2009). On the contrary, Haimanot (2002) found that *M. anisopliae* isolates were more virulent and produced a higher proportion of mycosised cadavers than the *B. bassiana* isolates but non-significant difference was in repellency test of termites with *M. anisopliae* and *B. bassiana* isolates under laboratory condition. The reason for their ineffectiveness might be due to fungal isolate or due to the development of defense mechanisms by termites against entomopathogenic fungi. A range of defense mechanisms against microbial epizootics has been reported in termites, including behavioral mechanisms (Rosengaus *et al.*, 1999), immune mechanisms (Rosengaus *et al.*, 2007) and antifungal chemical production in termite nests (Wright *et al.*, 2000). One such chemical is norharmane isolated from *Reticulitermes* sp. (Siderhust *et al.*, 2005). Singh and Singh (2002) demonstrated chlorpyrifos 0.04% was found most effective and gave 100% reduction in termite population up to five months in field condition.

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Genetic diversity in the assassin bug *Rhynocoris marginatus* Fabricius (Heteroptera: Reduviidae), based on RAPD analysis

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ABSTRACT: The present paper includes the results of RAPD-PCR based molecular genetic diversity of three populations of *Rhynocoris marginatus* Fabricius. The *in vitro* enzymatic amplification of genomic DNA derived from homogenized adult males and females was carried out by using three random oligonucleotide primers, respectively. The results were analysed by using standard gene ruler and by the application of suitable software programme by considering two parameters of study i.e., band sharing analysis and dendrogram of genetic relatedness. As a result of this research it was noticed that primer OPA-2 was most reliable because, on the basis of the DNA profiles with this primer, all the male and female of the populations of *R. marginatus* could be two main clusters and two subclusters together. The band sharing coefficient value of 1 indicated maximum similarity between all the three populations of *R. marginatus* with primer OPA-2. In this respect the results revealed in dendrogram and band sharing coefficient confirm the status of genetic kinship of inter- and intraspecific nature. © 2011 Association for Advancement of Entomology

KEYWORDS: Assassin bug, *Rhynocoris marginatus*, Genetic diversity, RAPD

INTRODUCTION

Assassin bugs are essential components of ecosystems, but also important in agriculture and medicine (Weirauch and Munro, 2009). Reduviid bugs often constitute a large

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and diverse group, have many fascinating specialized habits and as predators have considerable but unrealized potential as biocontrol agents (Ryckman and Ryckman, 1966; Ambrose, 1999). Among reduviids many species of the *R. marginatus* was reported as biocontrol potential predators of a number of insect pests of many crops of agricultural (Ambrose, 1999; Claver and Ambrose, 2003).

Putshkov (2002) argued that the genus *Rhynocoris* are very often manifest a significant degree of variability rather complicating the identification of species; it could be retained as a taxonomic unit for purely practical positions but it is very difficult to determine its genetic variability. The populations of *R. marginatus* that have been collected and are present in different regions of South India showed wide variations in morphological characteristics of head, antenna, rostrum, prothorax, wing, tibial pad, egg and abdomen (Ambrose, 1999, 2006).

The *R. marginatus* populations were named as Ayyanar Kovil Tropical Rainforest, Jamuna Mathur Scrub Jungle and Melmaruvathur Semiarid Zone, after their place of collection from where they were originally reported and were abundantly present. Due to over uses of pesticides and biopesticides in the filed situation of the *R. marginatus* populations are significantly reduce the egg hatching, nymphal development, nymphal mortality and predatory potential (Sahayaraj *et al.*, 2003).

In order to preserve the natural biodiversity present among *R. marginatus* populations, attempts are being made to understand the genetic structure of each population. Preliminary studies based on morphological, morphometrical, growth, biological, behavioral and biochemical etc. were made to understand the genetic basis of this phenotypic variability (Ambrose and Ambrose, 1996; Ambrose, 1999). However, no systematic and phylogenetic relationships studies were made to generate substantial information on the genetic diversity of *R. marginatus* populations so as to develop appropriate strategy for its conservation at the natural habitat.

Random amplified polymorphic DNA (RAPD) has emerged as one of the simplest, fastest and powerful tool for rapid identification of insect species (Garcia *et al.*, 1998; Pacheco *et al.*, 2003) as well as evaluating genetic diversity in filed collections (Margaritopoulos *et al.*, 2003; Sosa-Gomez *et al.*, 2004). The RAPD marker has made it possible to both distinguish between insect species (Dilawari *et al.*, 2005) as well as to appraise their genetic relatedness and hence hold unique significance in studies on insect populations and predatory potential in nearly real time situation (Li and Shen, 2002). Thus, in this study, we sought to establish a link between genetic diversity of *R. marginatus* using the three random oligonucleotide RAPD primers: this marker system was used to analyze the genetic variability among three populations of *R. marginatus*.

MATERIALS AND METHODS

Collection of *R. marginatus*

The adult males and females of *R. marginatus* were collected from Ayyanar Kovil Tropical Rainforest (AKTR) of Virudhunagar District, South Tamil Nadu, (altitude 399 M, latitude 76°39′E and 10°45′N), Jamuna Mathur Scrub Jungle (JMSJ) of

the Tiruvannamalai District, North Tamil Nadu (latitude 77°22′E and 8°11′N) and Melmaruvathur Semiarid Zone of the (MSZ) Kanchipuram District, North Tamil Nadu, India (latitude 77°31′E and 9°43′N). The AKTR is characterized by luxuriant growth of cover crops on the forest floor and a rich canopy. The JMSJ is a hilly terrain with small hillocks and boulders which cover the entire area. The MSZ is a dry semiarid habitat without hillocks and boulders but with boulders scattered around. They were reared in the laboratory under optimal conditions (temperature 28–35 °C, relative humidity 75–85% and photoperiod 11–13 h) on larvae of the rice moth *Corcyra cephalonica* Stainton. For the purpose of DNA extraction, freshly hatched adult male and female were put individually in eppendorf tubes, fixed in ethanol and stored at −70 °C.

DNA extraction

DNA was extracted from the legs and heads of *R. marginatus* without antennae and mouthparts (Rogers and Bendich, 1988). The severed legs and heads were added to 400 μl of extraction buffer (200 mM Tris-HCl (pH 8.0), 70 mM EDTA, 2 M NaCl and 1% of β-mercaptoethanol) and were crushed inside a microcentrifuge tube with a tissue homogenizer. After the addition of 160 μl of 5% CTAB, the samples were kept at 65°C for 5 min, following homogenization. An additional incubation at 60°C for 60 min was performed after adding 5.6 μl of 10 mg/ml proteinase K. After that, samples were centrifuged at 14000 rpm for 15 min, the aqueous phase was recovered (400 μl) and transferred to a new microcentrifuge tube, to which the equal volume of chloroform/isoamyl alcohol (24:1) was added. The DNA was precipitated following addition of 45% of the volume of 10 M NH_4OAc and equal volume of isopropanol. These tubes were kept overnight at −20°C or 2 h at −80°C. Samples were centrifuged at 14000 rpm for 15 min, the supernatant was discarded and the pellets were washed with 300 μl of 70% ethanol. The pellets were dried, with the tubes inverted on the bench at room temperature for one hour.

The pellets were then resuspended with 100 μl TE buffer with RNAse at final concentration of 10 μg/ml and kept at 37°C for 30 min. DNA aliquots kept at −20°C were thawed when necessary to perform the DNA amplification.

DNA amplification

The genomic DNA from *R. marginatus* of male and female collected from three different habitats were subjected to PCR using three different random oligonucleotides primers having base composition of 5′-GTTTCGTCC-3′ (OPA-1), 5′-GTAGACCCGT-3′ (OPA-2) and 5′-AAGAGCCCGT-3′ (OPA-3) (Borges *et al.*, 2000: Vargas *et al.*, 2006) obtained from Sigma-Aldrich (www.sigmaaldrich.com). The amplification reactions were conducted in a volume of 25 μl containing approximately 9 ng of template DNA, an appropriate amount of Milli Q sterile water, 10X buffer, 0.4 μM primer, 2.4 mM $MgCl_2$, 0.1 mM of each dNTP and *Taq* polymerase enzyme (Gibco BRE) (1U) per PCR tube amplification. Immediately after adding the reaction mixture, DNA was amplified. Reactions were performed using a PTC 200

gradient thermal cycler (MJ Research, Inc., www.mjr.com). The PCR programmed for 4 min at 94°C for initial denaturation. Following the initial denaturation the thermal cycler was programmed for 40 cycles of 1 min at 94°C for denaturation, 1 min at 52°C for annealing and 1 min at 72°C for extension. An additional cycle of 5 min at 72°C was also used for primer extension. The same DNA, magnesium, dNTP and primer concentrations were used in the reactions to avoid reproductive problems and the samples were amplified in the same thermal cycler using the same programme (Liu *et al.*, 1999).

Electrophoresis of PCR products

The amplified PCR products obtained in this way were resolved on 1% agarose gel containing ethidium bromide with a concentration of 0.5 mg/ml using 1X TAE buffer (40 mM Tris/acetate, 1 mM EDTA, pH 8.0) at a constant voltage of 50 V. DNA bands were visualized under UV light and were scored for their presence or absence using a binomial code (1/0). The molecular weight of each band was estimated by comparison to a co-migrating 100 bp DNA ladder (Fermentas Inc., www.fermentas.com).

Band sharing analysis

The band sharing coefficient helps to determine the similarity between individuals. Depending upon the presence or absence of the DNA fragments, the data was scored as 1 for the band present and 0 for its absence. Accordingly the band sharing coefficient between individual was calculated by employing the following formula which is based on the principle that the increase in the value of band sharing coefficient increases the genetic diversity among the members of the different three populations of *R. marginatus*.

$$D = 2N_{ab}/(N_a + N_b)$$

D = Band sharing coefficient

N_ab = Number of bands shared by individuals a and b

N_a = Number of bands obtained by a

N_b = Number of bands obtained by b

Dendrogram

A dendrogram showing the relationship between *R. marginatus* populations were generated by hierarchical cluster analysis. A phylogenetic analysis was undertaken using single linkage method and a dendrogram was constructed with the three primers. Genetic distance matrices were computed with SIMGEND program and the dendrogram was generated with the SAHN program using the UPGMA of the NTSYS-PC software (Rholf, 1993).

RESULTS AND DISCUSSION

The extraction of genomic DNA from an individual *R. marginatus* by the method described above gave reproducible results and provided sufficient amount of DNA

subsequent protocols. The concentration of DNA as determined by spectrophotometric analysis ranged between 0.25–0.45 $\mu g/\mu l$ for different samples. The use of the extracted *R. marginatus* DNA samples in the PCR amplification (RAPD) by using all the three primers of arbitrary sequence resulted in a series of discrete DNA fragments on agarose gel electrophoresis.

RAPD profile of *R. marginatus* from Ayyanar Kovil Tropical Rainforest

With primer OPA-1, a total of 3 and 4 recognizable bands were generated in the genomic DNA of male and female respectively in the tropical rainforest population of *R. marginatus* with the size of these bands ranging from 180–820 bp. Likewise, with primer OPA-2, a total of 1 and 2 bands were observed in the size range of 160–800 bp for male and female respectively. With primer OPA-3, a total of 4 and 4 bands in the size range of 190–800 bp were observed for male and female respectively (Fig. 1A).

RAPD profile of *R. marginatus* from Jamuna Mathur Scrub Jungle

With primer OPA-1, a total of 4 and 4 bands were observed for the male and female respectively in the scrub jungle population of *R. marginatus* with the size of these bands ranging from 180–820 bp. When OPA-2 was used, only one band was observed in the size range of 160–370 bp for both male and female respectively. With primer OPA-3, a total of 3 and 2 bands in the size range of 190–860 bp were observed for male and female respectively (Fig. 1B).

RAPD profile of *R. marginatus* from Melmaruvathur Semiarid Zone

When primer OPA-1 was used, a total of 3 and 3 bands were observed for the male and female respectively in the semiarid zone population of *R. marginatus* with the size of these bands ranging from 180–820 bp. In the case of primer OPA-2 only one band was observed in the size range of 160–640 bp for both male and female respectively. With primer OPA-3, a total of 2 and 2 bands in the size range of 190–860 bp were observed for male and female respectively (Fig. 1C).

There are three random primers that generated highly reproducible RAPD bands, with the highest of 18 bands in tropical rainforest ecotype, 15 bands in scrub jungle ecotype and 12 bands in semiarid zone ecotype of *R. marginatus*. Among the three ecotypic populations the total number of bands detected was lower for semiarid zone ecotype. The present study suggested that the differences in DNA bands among the three ecotypic *R. marginatus* populations might be due to the variation of different ecotypic factors. The present observations corroborate the finding of Ryman *et al.* (1995), Chaudhry and Sharma (2006) and Vargas *et al.* (2006) that different ecotypic populations of the species can be attributed to the matching genetic and physiological changes as reflected on the molecular characterization of the DNA bands.

Band sharing analysis

The value of the band sharing coefficient was used to assess the genetic diversity of *R. marginatus* populations. A band sharing coefficient value of 1 indicates maximum

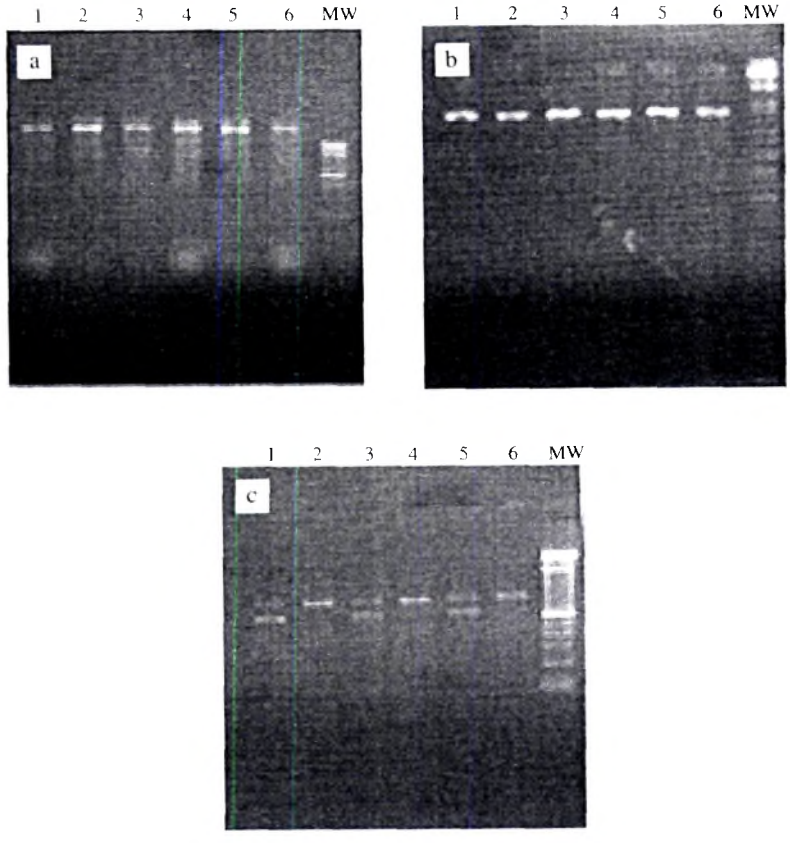


FIGURE 1. Agarose gel electrophoresis (1%) of the RAPD-PCR amplicon of assassin bug *R. marginatus* with three primers OPA-1, OPA-2 and OPA-3. Representative gel of three experiments male and female of *R. marginatus* (n = 3 each) from three different ecotypic populations (tropical rainforest-TR, scrub jungle-SJ and semiarid zone-SA) were analyzed with the above different primers. Lane 1 and 2: *R. marginatus* male and female (TR population). Lane 3 and 4: *R. marginatus* male and female (SJ population). Lane 5 and 6: *R. marginatus* male and female (SA population). Lane MW: 100 bp DNA ladder.

genetic similarity across the three ecotypic populations with primers OPA-1, OPA-2 and OPA-3 (Table 1). Based on the analysis of these bands, it was observed that there was no genetic difference between the members of the three populations of *R. marginatus* when primer OPA-2 was used. In addition to this, the band sharing coefficient between individual was also calculated. Based on the number of bands shared by all the three populations of *R. marginatus*, it was found that the females of all the populations had close genetic homologies. With primer OPA-1 and OPA-2, the female populations of tropical rainforest and scrub jungle revealed 100%

similarity (Table 1). Thus, different ecotypic genetic diversity in the *R. marginatus* were observed in the present study and such different ecotypic genetic diversity were documented in triatomine reduviids such as *Triatoma venosa* Stål (Vargas *et al.*, 2006), *Panstrongylus megistus* Burmeister (Barbosa *et al.*, 2006), *Bemisia* (Brown *et al.*, 2006) and *Psammolestes tertius* Lent and Jurberg (Soares *et al.*, 2001) with the technique of RAPD.

Dendrogram

On the basis of the observed discrete DNA fragments statistical analysis was carried out to assess the extent of genetic diversity among the three populations of *R. marginatus*. In the present investigation, the homology and differences in the DNA bands generated by specific sequence, matching with the random primers, were taken into consideration. The frequency of occurrence of the amplified fragments of DNA were also analyzed using the Neighbour-joining analysis in which all the amplified fragments were scored as 'present' or 'absent' and were assigned a value of 1 and 0 respectively. In the present studies, UPGMA analysis was used to analyze the grouping between the insects of a specific group as well as to differentiate various populations of *R. marginatus*.

The dendrogram summarizes the genetic relationships among the *R. marginatus* populations built using the bands that were generated in the RAPD method with all the three primers. The values of genetic difference among clusters of individuals ranged from 0.01 to 0.117 and the highest value of 0.117 was found between the individuals of denoted female from semiarid zone population. The lowest value of 0.01 found between the individuals of male from scrub jungle and semiarid zone populations (Fig. 2).

A cluster analysis indicated that primer OAP-2 was most reliable because on the basis of the DNA profile with this primer, the semiarid zone populations of *R. marginatus* would be clustered separately among the three populations. Similarly, a dendrogram with primer OPA-1 and OPA-3 showed more similarity between tropical rainforest and scrub jungle populations of *R. marginatus* while semiarid zone population was not much closer to the other two populations. It was observed that the DNA sequences amplified with primer OPA-2 may not have sufficient taxonomic value. This observation is in agreement with the results of (Ballinger-Crabtree *et al.*, 1992) in which the DNA sequences generated with some of the random primers were of no taxonomic value for constructing the dendrogram through UPGMA analysis of genus *Aedes*. The ecotypes from different habitats as with presumable diverse environmental factors are probably responsible for the genetic diversity of the populations (Margaritopoulos *et al.*, 2003; Vargas *et al.*, 2006).

The results of RAPD analysis by dendrogram and band sharing analysis coefficient confirm the status of genetic kinship of inter- and intraspecific nature. It may be added that the tropical rainforest and scrub jungle populations of *R. marginatus* share some of the common environmental factors such as semi-hilly terrain with identical breeding, resting sites and sources of body fluid meal which are the characteristic features for

TABLE 1. Band sharing analysis between three ecotypes of assassin bug, *R. marginatus* as determined by band sharing coefficient.

Primers	Individuals Analyzed	Band Sharing Coefficient
OPA-1	*R. marginatus* (Male) and *R. marginatus* (Female) (TR population)	0.85
	R. marginatus (Male) and *R. marginatus* (Female) (SJ population)	1.00
	R. marginatus (Male) and *R. marginatus* (Female) (SA population)	0.88
	R. marginatus (Female) (TR population) and *R. marginatus* (Female) (SJ population)	1.00
	R. marginatus (Female) (SJ population) and *R. marginatus* (Female) (TR population)	1.00
	R. marginatus (Female) (SJ population) and *R. marginatus* (Female) (SA population)	0.88
OPA-2	*R. marginatus* (Male) and *R. marginatus* (Female) (TR population)	0.66
	R. marginatus (Male) and *R. marginatus* (Female) (SJ population)	1.00
	R. marginatus (Male) and *R. marginatus* (Female) (SA population)	1.00
	R. marginatus (Female) (TR population) and *R. marginatus* (Female) (SJ population)	1.00
	R. marginatus (Female) (SJ population) and *R. marginatus* (Female) (TR population)	1.00
	R. marginatus (Female) (SJ population) and *R. marginatus* (Female) (SA population)	1.00
OPA-3	*R. marginatus* (Male) and *R. marginatus* (Female) (TR population)	1.00
	R. marginatus (Male) and *R. marginatus* (Female) (SJ population)	0.66
	R. marginatus (Male) and *R. marginatus* (Female) (SA population)	0.66
	R. marginatus (Female) (TR population) and *R. marginatus* (Female) (SJ population)	1.00
	R. marginatus (Female) (SJ population) and *R. marginatus* (Female) (TR population)	1.00
	R. marginatus (Female) (SJ population) and *R. marginatus* (Female) (SA population)	0.66

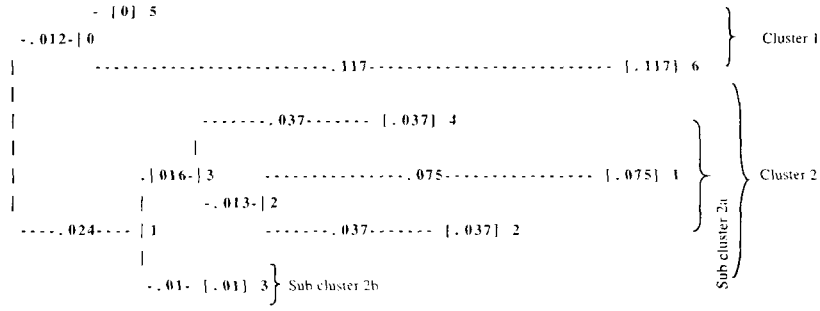


FIGURE 2. Dendrogram was constructed according to UPGMA method for three different ecotypes p (tropical rainforest-TR, scrub jungle-SJ and semiarid zone-SA) of assassin bug *R. marginatus* using three primers OPA-1, OPA-2 and OAP-3. Cluster: 1 *R. marginatus* male (5) and female (6) (SA population); cluster: 2 *R. marginatus* male (1) and female (2) (TR population); male (3) and female (4) (SJ population); sub cluster: 2a *R. marginatus* female (4) (SJ population); male (1) and female (2) (TR population) and sub cluster: 2b *R. marginatus* male (3) (SJ population).

tropical rainforest and scrub jungle populations of *R. marginatus*. As compared to this, semiarid zone population with different environmental factors have entirely different mode of breeding and feeding due to which the species is known more as a tropical rainforest and scrub jungle populations than semiarid zone population. The bionomics of the species can be attributed to matching the genetic and physiological changes are reflected here in the study of the molecular characteristics of the genome of this species and hence is of immense epidemiological significance (Chaudhry and Sharma, 2006).

No molecular data are available to the public database till date on the genetic diversity of *R. marginatus*. These results are some of the preliminary finding which will assist in future research on this subject and may be suitable for more detailed molecular analysis. In our study we have demonstrated the RAPD analysis may be useful not only to discriminate genotypes among populations, but also shed light on the understanding of the genetic diversity among the three ecotypes namely tropical rainforest, scrub jungle and semiarid zone in South India.

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Functional response of *Canthecona furcellata* Wolff. (Hemiptera: Pentatomidae) to tropical Tasar silkworm *Antheraea mylitta* Drury (Lepidoptera: Saturniidae)

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ABSTRACT: Predacious stink bug, *Canthecona furcellata* Wolff. evinced Holling's Type II curvilinear functional response to the prey tropical tasar silk worm, *Antheraea mylitta* Drury. The predator's attack rate increased as the prey density increased which was confirmed by positive correlation. The maximum predation ($k = 5.90$) and rate of discovery (0.36) were found at higher prey density. Decreasing handling time (0.68) of prey led to increase attack rate by predator. The attack ratio (0.18) and searching time (1.14) by predator decreased with the increase in prey density which was further confirmed by the negative correlations obtained. At high prey density, predator spent no time in searching of prey therefore it spent more time in handling whereas at low prey density, the searching time was always observed higher than handling time.
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KEYWORDS: Attack ratio, functional response, *Canthecona furcellata* Wolff, *Antheraea mylitta* Drury, handling time

INTRODUCTION

The functional response defines the rate of prey consumption by a given number of predators as a function of prey density and therefore can predict the maximum number of prey that can be consumed by a given predator per day. The numerical models (Type I, II and III) were also formulated to describe predatory responses which were influenced by changes in predator behavior (Holling, 1959). Predators development, survival and reproduction can be effected by the number of prey

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consumed (Oaten and Murdoch, 1975). Functional responses of a predator is a key factor regulating population dynamics of prey – predator system and determining the effects of predations on prey population (Huffaker and Messenger, 1976; Mandour *et al.*, 2006).

Stink bug, *Canthecona furcellata* Wolff. (Hemiptera: Pentatomidae) frequently occurs as a potent predator of insect pests of many Lepidopterous species like *Eutectona machaeralis* (Pyralidae); *Hybloea puera* (Hyblaeidae); *Nephopteryx rhodobasalis* (Pryalidae), *Plecoptera reflex* (Noctuidae), *Clostera cupreata* (Notodontidae), *Spodoptera litura* Fabr. (Noctuidae), *Helicoverpa armigera* (Noctuidae), *Amsacta albistriga* (Arctiidae) and *Cnaphalocrocis medinalis* Linn. (Pyralididae) (Beeson, 1941; Kumar and Singh, 2007). Flower feeder of weed (*Parthenium* sp.) *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) was also predated by *C. furcellata* (Singh *et al.*, 2009).

Tropical tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae) rearing being out doored is exposed to the attack of predators and parasites inflicting cocoon crop loss of 40–50%. Alone *C. furcellata* is found to cause 13–15% damage to the larvae of *A. mylitta* D from July to November (Sen and Jolly, 1967; Sen *et al.*, 1971). Although there has been few reports on biology and predatory potential (Jolly, 1967; Sharma *et al.*, 2003) of *C. furcellata*, information on functional response of this bug against *A. mylitta* D. is still lacking. The aim of this work were to elucidate, via laboratory tests, the functional response of the predator to the density of *A. mylitta* of non mulberry sericulture industrial importance and to assess the utility of the results for formulating the management strategies to avert predation in tasar silkworm cocoon production.

MATERIALS AND METHODS

Culture of predator *C. furcellata* Wolff. was maintained on disease free larvae of prey, *A. mylitta* Drury reared on the foliage of arjuna host plant, *Terminalia arjuna* twigs in glass jars kept in indoor rearing room (25–30°C, RH 75–80% and 11–13 hrs photoperiod) of Regional Tasar Research Station, Warangal, Andhra Pradesh. Fresh larvae along with arjuna twigs were provided *ad libitum* as feed to predators.

A third instar larva of *A. mylitta* Drury was collected from field rearing plots and released on fresh arjuna host plant branches (1.20 mts length) placed in pitcher pot filled with water. Pot opening was covered with paddy straw bundle to avoid the larval movement into the pot. After 10 minutes, the predators were introduced on branches at densities of 1, 2, 4, 8, 16 and 32 prey/predator. The branches were loosely covered with nylon net bags (1.00 × 0.5 mtrs) after release of test insects. This experimental set up was maintained with six replicates for each prey density kept in indoor rearing room. Number of preys killed were counted and replaced by live ones along with fresh branches of host plant at 24 hr. interval for four days.

TABLE 1. Functional response of *C. furcellata* W. to *A. myllita* D. ($n = 4$)

Prey density (x)	Prey attacked (y)	Max. y (K)	Days per 'y' $(b) = Tt/k$	Days all 'y's (by)	Days searching $Ts = Tt - by$	Attack ratio (y/x)	Rate of discovery $(Y/x/Ts) = (a)$	'DISC' Equation $y = a(Tt - by)x$
1	0.80±0.39*			0.54	3.46	0.80	0.23	0.80
2	1.20±0.57*			0.82	3.18	0.60	0.19	1.21
4	2.60±0.80	5.90	0.68	1.77	2.23	0.65	0.29	2.59
8	3.80±1.17*			2.58	1.42	0.48	0.34	3.86
16	4.20±1.23*			2.86	1.14	0.27	0.36	4.38
32	5.90±1.52*			4.01	0.00	0.18	–	–
							Mean = 0.26	

(*) Significant at 5% level

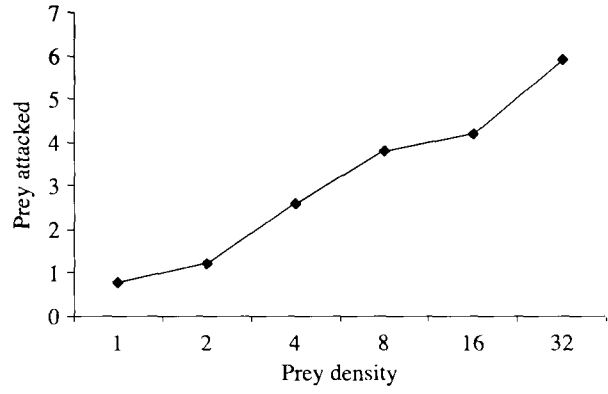


FIGURE 1. Functional response of *C. furcellata* to *A. mylitta D.*

Holling (1959) 'disc' equation was applied to describe the functional response of *C. furcellata* to *A. mylitta* as the descriptions given by Ambrose and Claver (1995); Claver *et al.* (2002). The data were subjected to linear regression analysis to determine the relationship between the prey density and the number of prey consumed, searching time and attack ratio.

RESULTS AND DISCUSSION

Table 1 shows the adult *C. furcellata* responded to the increasing prey density *A. mylitta* by killing more number of prey than prey killed at lower densities, thus exhibiting type II curvilinear functional response of Holling (1959). The number of prey *A. mylitta* killed y by the individual predator, *C. furcellata* increased as the prey density x was increased from one prey to 32 preys per predator. This was further conrmed by the positive correlation obtained between the prey density and the prey killed (Y 1 508 0.1501, x r 0 9201). The predation rate showed a steep rise from 1 to 16 prey densities and a plateau was maintained at 32 prey density (Fig. 1). Stabilization of predation is often attributed to a positive relationship between prey density and predation rate. Accelerating functional responses are the key characteristics of generalist predators which can result in density – dependent predation (Murdoch and Qaten, 1975). Holling (1959) observed that accelerating functional responses can also result from predators switching among alternative prey types, selectively foraging in areas of high density or learning from past experience. Similar type of response was also seen in some reduviid predators *Rhynocoris fuscipes* (Ambrose and Claver, 1995) against pod sucking bug, *Riptortus clavatus* and cucumber leaf folder, *Diaphania indicus* (Nagarajan *et al.*, 2010).

The maximum predation represented by 'k' value (5.90) was always found restricted to the higher prey density (32) of *A. mylitta*, because higher prey density enabled the predators to spend less time in search of food and to make use of all its time in

attacking and consuming the prey. Fig. 1 depicts a steep rise in predation at 1 to 16 prey density and showing Hollings type II curve. Such type-II curve is commonly found (Morris, 1963) in heteropteran predators. Predators *R. marginatus* (Ambrose and Claver, 1996) and *R. longifrons* (Claver *et al.*, 2002) too spent no time to search and utilized all their time to consume preys *Spodoptera litura* and *Clavigralla gibbosa* Spinola, respectively. Another important component of functional response is the attack rate. In *C. furcellata* the highest attack ratio (0.80) was observed at the density of one prey/predator and the lowest (0.18) at the density of 32 preys/predator, which was confirmed by negative correlation ($y = 0.3100 - 0.0181\ x : r = -0.9830$). The indirectly proportional relationship observed between the attack ratio (y/x) and prey density (X) was also similar as in case of *R. marginatus* (Ambrose and Claver, 1996), *R. longifrons* (Claver *et al.*, 2002), *R. fuscipes* (Nagarajan *et al.*, 2010) and *Nabis americoferus* (Propp, 1982). Increased prey consumption at higher prey density might also be attributed to phenomenal changes that occurs simultaneously in searching time, prey density and predator satiation. *C. furcellata* took 0.68 min to handle a *A. mylitta* larva (Table 1). Increase in handling time, an important component of functional response, leads to decreased attack rate (Holling, 1959). Handling time was also influenced by the starvation status of predator which increases the prey handling time (Ray, 2008). Small third instar larvae of *A. mylitta* were handled and consumed by *C. furcellata* in lesser time of experimental period. Flinn *et al.* (1985) reported that handling time is proportional to the size of the prey because predator takes a longer time to eat larger prey.

An interval between the consumption of a prey and the subsequent attack of the predator is known to be searching time (Holling, 1959). A negative correlation ($y = 0.8501 - 0.1012X$; $r = -0.9224$) obtained between the prey density and the searching time of the predator, *Canthecona* at all densities (Table 1) indicating the decelerate searching with the accelerate prey density of *Antheraea*. This was due to the lesser searching time at higher prey density which in turn might have caused a decline in the attack rate. In addition, satiation is a possible reason for decreased prey consumption at higher prey densities. Senrayan (1988) reported that *Eocanthecona furcellata* Wolff. spent less time for searching the prey, *Latoia lepida* Cramer at higher prey density. Earlier reports of Claver *et al.* (2002) and Nagarajan *et al.* (2010) corroborates the present findings and inferred that the probability of the predators higher prey contact at higher prey density would have enhanced the searching ability per unit area. The rate of discovery of the prey, *A. mylitta* increased as the prey density was increased in the present studies which was further confirmed by the positive correlation. ($y = 0.24 \pm 0.003\ x : r = 0.72$). Similar positive correlation was obtained between prey density and the rate of discovery in *Rhynocoris marginatus* Fabricius to *Spodoptera litura* Fabricius (Ambrose and Claver, 1995) in *Acanthaspis siva* Distant to *Camponotus compressus* Fabricius. In contrast, negative correlation was obtained between prey density and rate of discovery in *R. fuscipes* (Fab.) to three pests of pigeon pea (Claver and Ambrose, 2002) and *R. longifrons* (Stal) to *Clavigralla gibbosa* Spinola (Claver *et al.*, 2002).

The present findings enable us to conclude that *Canthecona* bug has already attained harmful status in tasar silk industry as it serves as potent natural enemy of early larval instars of *A. mylitta*. Hence it is suggested to carry out studies on management tactics for *C. furcellata* with an aim to prevent predation of early instar larvae of *A. mylitta* D during rearing seasons.

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Screening of turmeric (*Curcuma longa* L.) germplasm for resistance to shoot borer (*Conogethes punctiferalis* Guen.) (Lepidoptera: Pyralidae), in Kerala, South India

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ABSTRACT: Nine hundred and fifteen accessions of turmeric (*Curcuma longa* L.) available at Indian Institute of Spices Research, Kozhikode (Kerala, India) were screened in the field against the shoot borer (*Conogethes punctiferalis* Guen.) for four consecutive years. All the accessions were susceptible to the pest infestation. Rating of accessions in relation to the level of pest infestation indicated that none of the accessions was rated as resistant, whereas, 34, 412, 456 and 13 accessions were rated as moderately resistant, moderately susceptible, susceptible and highly susceptible, respectively, to the pest. © 2011 Association for Advancement of Entomology

KEYWORDS: turmeric, *Curcuma longa*, shoot borer, *Conogethes punctiferalis*, resistance

India is the largest producer of turmeric (*Curcuma longa* L.) (Zingiberaecae), in the world producing about 792,980 tonnes annually from an area of about 180,960 hectares (NHB, 2011). There is renewed interest in production of turmeric due to various biological and medicinal properties of curcumin, the main constituent of turmeric. The shoot borer (*Conogethes punctiferalis* Guen.) (Lepidoptera: Pyralidae) is the most serious pest of the crop in India (Devasahayam and Koya, 2007). The adult moths of the shoot borer lay eggs on the tender leaf and the larvae that hatches out bore into the shoots and feed on the inner core resulting in dead hearts or withered shoots.

The present recommendation of spraying insecticides for the management of the shoot borer is undesirable due to the increasing use of turmeric in medicinal and culinary preparations and as a food colourant. Development of resistant varieties and their incorporation in IPM schedules is an eco-friendly alternative for management

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of the pest. Earlier attempts to screen turmeric lines to the shoot borer indicated that Dindigram Ca-2 (Sheila *et al.*, 1980) and Manuthy Local (Philip and Nair, 1981) were least susceptible to the pest.Velayudhan and Liji (2003) identified 22 accessions that were more tolerant to the pest.

Screening of 915 turmeric accessions including indigenous and exotic types and seedling progenies was conducted at the Experimental Farm of Indian Institute of Spices Research, at Peruvannamuzhi (11°34′ N, 75°48′ E) (Kerala, India) for four years during 2005–09. The turmeric accessions were raised in large cement pots (45 cm height, 45 cm diameter) and filled with potting mixture containing soil, sand and dried powdered cow dung in 1:1:1 ratio and maintained under open conditions, The pots were place randomly, maintaining a distance of 50 cm between the pots. Three replications were maintained for each accession. Rhizomes (20–25 g size) of each accession were planted during June in the centre of the pot which developed into a clump, containing several shoots. All standard agronomic practices, except plant protection with pesticides were followed for raising the plants. The number of damaged (dead hearts/shoots with bore hole) and healthy shoots were recorded in each clump during October–November each year when the symptoms of pest damage reached its peak. The mean per cent shoot damage in each accession was calculated pooling the years.

The data obtained was subjected to ANOVA using SPSS package after arcsine transformation. The mean and standard deviation of maximum per cent shoot damage in each accession irrespective of the year was taken for calculating pest susceptibility ratings as per the method of Bhumanavar *et al.* (1989) and adopted for screening ginger germplasm against shoot borer (Devasahayam *et al.*, 2010):

1. Highly resistant: No shoot damage
2. Moderately resistant: Less than Mean – 2 SD shoot damage
3. Moderately susceptible: Mean – 2 SD to Mean shoot damage
4. Susceptible: Mean to Mean + 2 SD shoot damage
5. Highly susceptible: More than Mean + 2 SD shoot damage

All the turmeric accessions screened were susceptible to the shoot borer infestation. The pest susceptibility ratings of different accessions of turmeric indicated that none of the accessions was resistant, whereas, 34, 412, 456 and 13 accessions were moderately resistant, moderately susceptible, susceptible and highly susceptible, respectively, to the pest (Tables 1 and 2). All the moderately resistant accessions were indigenous types and seedling progenies. Among the improved varieties released by IISR, Kozhikode, none were moderately resistant whereas, Suguna, Sudarshana, Suvarna, Prabha, Prathiba and Kedaram were moderately susceptible to the pest.

Since the variation in the per cent shoot damage between various accessions was wide, it was not possible to fix the upper and lower limits of each group as constant values. Hence, the mean and standard deviation of the maximum shoot damage irrespective of the year was used for calculating various categories of resistance/susceptibility. The categorization based on the extent of variation from

TABLE 1. Reaction of turmeric accessions to shoot borer (*Conogethes punctiferalis*)

Category of resistance	Range of shoot damage (%) (Criteria for classification)	No. of accessions
Resistant	0	0
Moderately resistant	< 13.57 (less than mean – 2 SD)	34
Moderately susceptible	13.58 – 28.95 (mean – 2 SD to mean)	412
Susceptible	28.96 – 44.33 (mean to mean + 2 SD)	456
Highly susceptible	>44.34 (more than mean + 2 SD)	13

Mean = 28.95; SD = 7.69.

TABLE 2. List of turmeric accessions exhibiting a moderately resistant reaction to shoot borer (*Conogethes punctiferalis*)

Category of resistance	Accession
Moderately resistant	954, 619, 1026, 422, 435, 589, 687, 579, 964, 1028, 751, 693, 592, 1036, 1039, 483, 749, 438, 931, 972, 684, 413, 433, 419, 615, 930, 580, 381, 594, 1010, 911, 985, 1034, 932

The accessions are arranged in ascending order of shoot damage.

the mean (positive or negative) reduced the probabilities of inclusion of pseudo-resistant/susceptible accessions (Bhumanavar *et al.*, 1989).

Wide variations in pest susceptibility was also reported while screening brinjal germpalsm for resistance against shoot and fruit borer (*Leucinodes orbonalis* Guen.) (Lal *et al.*, 1976) and ginger germplasm for resistance against shoot borer (*C. punctiferalis*) (Devasahayam *et al.*, 2010). Earlier screening studies by Sheila *et al.* (1980) and Philip and Nair (1981) were conducted for a single year and with a limited number of accessions (13 and 9 accessions, respectively) only. Though Velayudhan and Liji (2003) screened 489 accessions, their study was limited to a single year only. The present study was conducted for four years, and the methodology adopted for rating the accessions also overcomes the variations in pest susceptibility during years. The 34 accessions which exhibited a moderately resistant reaction to the shoot borer in the present study provides a wide choice of breeding lines for developing turmeric varieties resistant to the shoot borer.

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Alphitobius Stephens (Coleoptera: Tenebrionidae: Tenebrioninae: Alphitobiini) from south India

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ABSTRACT: First report of the beetle, *Alphitobius diaperinus* (the lesser mealworm), a serious pest of poultry farms from the western slopes of the moist south Western Ghats in South India, detailed description of the two species *Alphitobius diaperinus* and *A. laevigatus* (the black fungus beetle), distributional pattern of the genus and a pictorial key to the species are provided. © 2011 Association for Advancement of Entomology

KEYWORDS: Lesser mealworm, black fungus beetle, poultry pest.

INTRODUCTION

Tribe Alphitobiini Reitter, 1917 (Coleoptera, Tenebrionidae, Tenebrioninae) is represented by seven genera namely *Alphitobius* Stephens, 1829, *Diaclina* Jacquelin du Val, 1861, *Metaclisa* Jacquelin du Val, 1861, *Hoplopeltis* Fairmaire, 1894, *Peltoides* Laporte, 1832, *Guanobius* Grimm, 2008 and *Alphitopsis* Kirejtshuk et al., 2012 from the Palaearctic, Nearctic, Oriental, Ethiopian, Australian and Neotropical regions (Doyen, 1989; Doyen et al., 1989; Aalbu et al., 2002; Löbl et al., 2008; Grimm, 2008; Kirejtshuk et al., 2012). Only two genera of the tribe namely, *Diaclina* and *Alphitobius* are represented from India (Kaszab, 1979). Among these two Indian genera, *Alphitobius* is represented by two species in India namely, *Alphitobius diaperinus* and *A. laevigatus*. *Alphitobius diaperinus*, commonly known as "the lesser mealworm" is a common pest of poultry houses and broiler sheds, transmitting a large number of poultry diseases and is difficult to be controlled by conventional means (Dunford and Kaufman, 2006). *Alphitobius laevigatus* is commonly called "the black fungus beetle" and found more often in garden refuse or near trees (Dunford and Kaufman, 2006). Except for the record of *A. laevigatus* from the western slopes of the moist south Western Ghats (Kaszab, 1979) and of *A. diaperinus* from the dry eastern slopes of the Western Ghats (Karunamoorthy et al., 1994), no information on the genus exists in south India. In the

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present work, first report of the species *A. diaperinus* from the western slopes of the moist south Western Ghats in south India and a modified pictorial key to the species of the genus *Alphitobius* from the region is provided. Since the original descriptions were brief, detailed redescriptions are provided for *A. diaperinus* and *A. laevigatus* along with the regional distribution pattern.

MATERIALS AND METHODS

Abbreviations of specimen depositories

NPC National Pusa Collection, Indian Agricultural Research Institute, New Delhi, India.

SJC St. Joseph's College, Devagiri, Calicut, Kerala, India.

ZSI Zoological Survey of India, Calicut, Kerala, India.

Specimens were collected with light traps from residential buildings, pit fall traps from the agricultural fields and natural forests, by hand picking from below stones and dung pats, among bedding material (wood saw) in poultry farms and natural forests during 2004 to 2011. Identification up to genus level was done with the keys available in Mathews and Bouchard (2008) and up to species level by following the key provided by Brendell (1975) but the keys provided earlier were not pictorial which makes species level identification difficult. Identification has been confirmed by comparing with the verified specimens in the coleoptera collections of the Hungarian Natural History Museum. Line drawings of structures for the pictorial key were made using Camera Lucida. Body length was measured from the anterior margin of the pronotum to the apex of elytra (Merkkl and Kompantzeva, 1996). All measurements are in millimeters.

Abbreviations of measurements

TL	length from the anterior margin of the pronotum to the apex of elytra;
TW	maximum body width;
PL	length of pronotum along median line;
PW	greatest width of pronotum;
EL	length of elytra.

Alphitobius Stephens, 1829

Alphitobius Stephens. Type species: *Helops picipes* Panzer (*Opatrum laevigatum* Fabricius), by monotypy; *Microphyes* Macleay. Type species: *Microphyes rufipes* Macleay, by monotypy. Synonymy by Blair. Mathews and Bouchard, 2008:165.

Color. Brownish black, ventral surface reddish brown.

Head. Edge of the clypeus shallowly emarginate. Eyes transverse, reniform, divided by frontal canthus. Labrum transverse, largely covered by clypeus.

Pronotum. Transverse, widest basally, evenly convex.

Elytra. With nine striae and scutellaty striole, epipleura complete to apex.

Venter. Prosternum moderately long before coxae. Prosternal process acutely prolonged and received in deep V-shaped concavity of the mesoventrite. Mesocoxal cavities closed by ventrites only.

Legs. Not fossorial, tarsomeres not lobed, plantar surfaces covered with sparse bristles.

Male Genitalia. Aedeagus sclerotised, proximal end curved asymmetrically.

Geographic distribution. Cosmopolitan, reported from the US, Europe, Africa, Asia and the Mediterranean.

(Modifie from Brendell 1975). (All scales 1 mm.)

Alphitobius diaperinus (Panzer, 1797)

Alphitobius diaperinus



Tenebrio diaperinus Panzer, 1797:16; *Phaleria diaperinus* Panzer and Latreille 1804; *Uloma opatroides* Dejean, 1821; *Uloma mauritanica* Curtis, 1831; *Alphitobius mauritanicus* Curtis, Stephens 1832; *Heterophaga opatroides* Dejean, 1833;

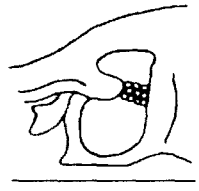
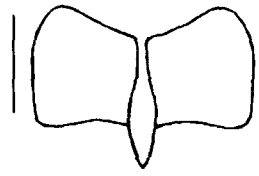
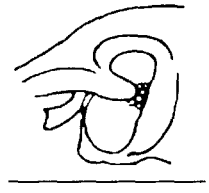
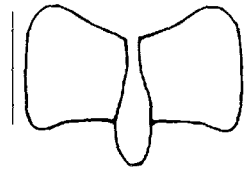
1	(a) Eyes emarginate with 3–4 ommatidia wide at its narrowest point 	(b) Prosternal process pointed apically. 	*Alphitobius diaperinus*
2	(c) Eyes deeply emarginate with 1-2 ommatidia wide at its narrowest point 	(d) Prosternal process rounded apically. 	*Alphitobius laevigatus*

FIGURE 1. Pictorial key to species of the genus *Alphitobius* Stephens from south India.

Heterophaga diaperina Panzer, Redtenbacher 1849; *Alphitobius diaperinus* Panzer, Wollaston 1854; *Helops picipes* Panzer, 1794 (*Opatrum laevigatum* Fabricius, 1781), by monotypy; Synonymy by Blair 1914:486.

Body. Broadly oval, moderately convex.

Color. Shining dark brownish black.

Head. Emarginate in front, with a distinct clypeal groove, the surface coarsely punctured. Antennal segments 5–10 assymetrically enlarged to the apex. Eyes emarginated with the narrowest point 3–4 ommatidia wide.

Pronotum. Pronotal disk nely and sparsely punctured, with punctures much coarser laterally.

Elytra. Elytra with moderately impressed striae having finely punctured, feebly convex intervals. Elytral punctures sparse and nearly as large as those of the striae.

Venter. Ventral surface reddish-brown, with the prosternal process horizontal between coxae.

Aedeagus. Basal piece longer than apical, parameres fully fused.

Measurements (in mm; 16 males & 27 females). TL = 5.8–6.3, EL = 3.1–3.3, PL = 1.9–2.1, PW = 1.9–2.2, EL = 3.7–3.9.

Materials Examined. (*n* = 65) INDIA. Kerala: Arakulam (230 MASL, 11° 26.528′ N; 76° 15.063′ E), Poultry excreta, hand picking, 10.V. 2007, T. K. Sabu (4 males, 12 females, SJC; 1 male, 3 female, ZSI; 1 male, 1 female, NPC); Tachampara (96 MASL, 10° 56.828′ N; 76° 30.611′ E), Poultry excreta, hand picking, 06.XII. 2009, C. Arunraj (2 males, 6 females. SJC; 2 male, 2 female. ZSI; 3 male, 2 female. NPC); Periya (800 MASL, 11° 26.225′ N; 76° 15.169′ E), Elephant dung, 12.I.2004, K V. Vinod (1 male. SJC); Thirunelli (950 MASL, 11° 54.649′ N; 75° 59.722′E), Elephant dung 5.II.2004. K. V. Vinod (1 female. SJC); Kattikulam (787 MASL, 11° 50.881′N; 76° 03.797′E), Elephant dung, 05.II.2003, K. V. Vinod (2 males, SJC); Dindigul (279 MASL, 10° 23.494′ N; 78° 02.856′E), Poultry farm, 12.V.2011, C. Arunraj (10 males, 12 females. SJC).

Habits. The specimens were collected from elephant dung, forest litter and poultry dung brought into the agriculture fields from the eastern slopes of the Western Ghats (Pollachi and Dindigul in Tamil Nadu)

Distribution in south India. Agricultural belts in the south Western Ghats and Montane evergreen forests in the Wayanad region of the Western Ghats.

Alphitobius laevigatus (Fabricius, 1781). *Opatrum laevigatum* Fabricius, 1781:90; *Microphyes rufipes* Macleay, 1872:286).

Body. Broadly oval, flat.

Color. Dark Brownish black.

Head. Deeply emarginate in front, with a distinct clypeal groove, the surface coarsely punctured. Antennal segments 6–10 assymetrically enlarged to the apex. Eyes emarginated with the narrowest point 1–2 ommatidia wide.

Pronotum. Pronotal disk corsely punctured, with punctures even.

Elytra. Elytra with moderately impressed striae with finely punctured, feebly convex intervals. Elytral punctures sparse and nearly as large as those of the striae.

Venter. Ventral surface dark reddish-brown, with prosternal process convex between coxae.

Aedeagus. Slender with parameres laterally separate.

Measurements. (3 males & 18 females). TL = 4.5–6.25, EW = 3.1–3.3, PL = 1.8–2.1, PW = 1.9–2.2, EL = 3.6–3.9.

Materials Examined. (n = 21) INDIA. Kerala: Chinnar (488 MASL, 10° 20.913′ N; 77° 13.422′ E), forests; light attracted specimen, 29.VII. 2010 & 11.V.2011, C. Arunraj (21, SJC, ZSI, and NPC).

Habits. Light attracted specimen in forest and no other details are available.

Distribution in south India. Coimbatore (Kaszab, 1979) and dry deciduous forests at Chinnar in the eastern slope of the South Western Ghats.

Diagnosis. Shape of the prosternal intercoxal process is a formerly unnoticed character that can be used for the differentiation of *A. diaperinus* & *A. laevigatus*. Intercoxal process of *A. diaperinus* is pointed in contrast to the rounded process in *A. laevigatus*. The shape of the fore tibia is often considered as a character for the species level differentiation of *Alphitobius* (Brendell, 1975). The apex of the fore tibia is broader than that of the base in *A. diaperinus* in contrast to the uniform breadth of the base and apex in of the fore tibia in *A. laevigatus*. However, intraspecies variation in the shape of the fore tibia in the female of *A. laevigatus* would lead to its wrong diagnosis as*A. diaperinus* making it a less dependable character.

Remarks. The present effort reveals that *Alphitobius diaperinus* is common in the poultry dung reaching the western slopes of south Western Ghats from the dry eastern slopes in addition to its record from the Wayanad forests in the moist south Western Ghats whereas *A. laevigatus* is reported from the forests in the dry eastern and moist western slopes of the Western Ghats.

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Description of two new aleyrodids of the genus *Dialeurodes* Cockerell (Aleyrodidae: Hemiptera) from India

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ABSTRACT: Two new species of the whitefly genus *Dialeurodes* Cockerell viz., *D. loganiacei* sp. nov. on *Strychnos wallichiana* and *D. trilobiata* sp. nov. on *Syzygium* sp., *Morinda pubescens* and *Cinnamomum malabathrum* are described and illustrated.
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KEYWORDS: Aleyrodidae, *Dialeurodes*

INTRODUCTION

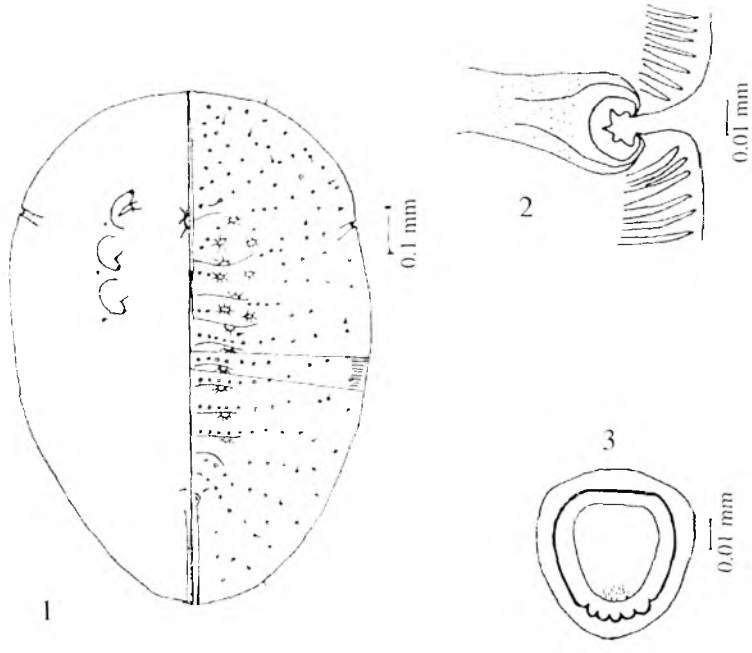
The whitefly genus *Dialeurodes* Cockerell was represented in India by 15 species (Sundararaj and Dubey, 2006). Martin and Mound (2007) synonymised *Kanakarajiella* David & Sundararaj and *Shanthiniae* David with *Dialeurodes* Cockerell and *Dialeuronomada* Quaintance & Baker and *Rabdostigma* Quaintance & Baker as subgenera under *Dialeurodes* Cockerell with the note that future studies may reveal some or all of these to be valid as full genera. Sundararaj and Pushpa (2011) reinstated the generic status of *Kanakarajiella*, *Dialeuronomada* and *Rabdostigma*. Thus the genus *Dialeurodes* is represented by 20 species in India. In this paper two species of *Dialeurodes* viz., *D. loganiacei* sp. nov. on *Strychnos wallichiana* and *D. trilobiata* sp. nov. on *Syzygium* sp., *Morinda pubescens* and *Cinnamomum malabathrum* are described and illustrated.

MATERIALS AND METHODS

The present finding was largely based on the whitefly materials collected from various localities of Western Ghats of south India during the period 2005–09. The whitefly infested leaves were collected from the host plants and permanent mounts of the

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FIGURES 1–3. *Dialeurodes loganiacei* sp. nov. 1. Puparium; 2. margin with thoracic tracheal fold; 3. vasiform orifice.

puparia were prepared by adopting the method suggested by David and Subramaniam (1976). Observations, micro-measurements and camera lucida drawings were made by using Nikon Optiphot T-2 EFD microscope and the identity of the whitefly was confirmed.

RESULTS

Description

1. *Dialeurodes loganiacei* sp. nov. (Figs. 1–3)

Puparium (Fig. 1): White, without wax; oval, broadest at the transverse moulting suture, narrowed in the posterior end, 1.56–1.92 mm long, 1.08–1.40 mm wide; found in groups on the under surface of leaves. Margin smooth, thoracic and caudal tracheal pore regions indicated as invaginated clefts with chitinized rim having irregular teeth (Fig. 2). Anterior and posterior marginal setae each 12 μm long.

Dorsum: Submargin with striations; subdorsum with irregular striations, many transverse rows of round pore-like papillae on dorsum. Submedian pockets present in all segment sutures, submedian depressions discernible on all the segments. Longitudinal moulting suture reaching margin and transverse moulting suture reaching submedian area. Minute pores and porettes scattered throughout the dorsum. Thoracic tracheal furrows indicated. Caudal tracheal furrow 200–292 μm long, 30–40 μm wide at its widest end.

Chaetotaxy: Four pairs of setae-cephalic setae 26–30 μm long, first abdominal setae 25 μm long, eighth abdominal setae cephalolaterad of vasiform orifice 10 μm long and caudal setae 14 μm long. Subdorsum with a row of 13 pairs of minute setae, each 2.5 μm long.

Vasiform orifice (Fig. 3): Subcircular, as long as wide, (52–60 μm), posterior wall of orifice, with a comb of inner teeth; operculum subcordate, broader at the anterior, slightly constricted about its middle and narrows posteriorly, 40–42 μm long, 44–46 μm wide, filling the orifice, lingula concealed.

Venter: Paired ventral abdominal setae 20 μm long, 60 μm apart. Spiracles visible at the base of legs. Thoracic and caudal tracheal folds distinct with stipples. Antennae reaching base of prothoracic legs.

Specimens examined

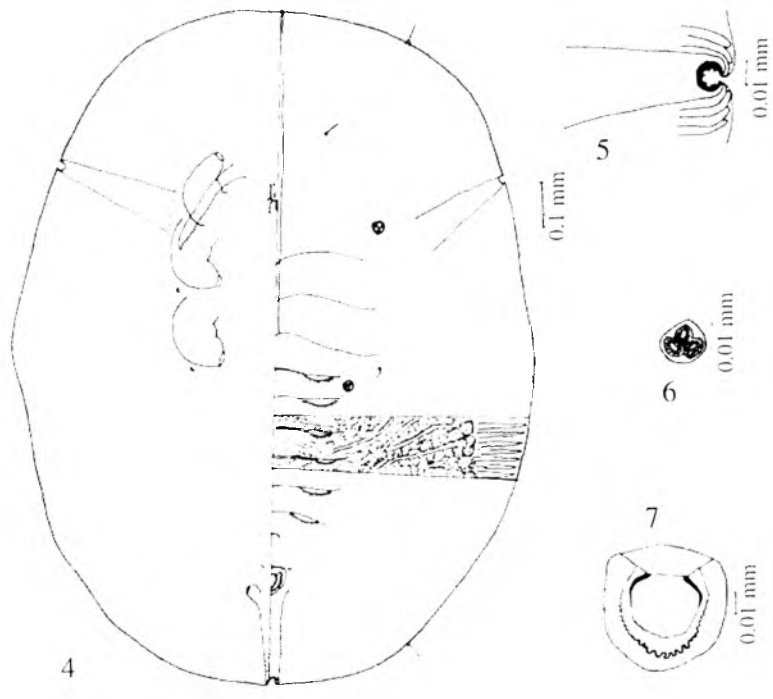
Holotype: puparium, India: Kerala: Karadipara (Nelliyampathi), on *Strychnos wallichiana*, 23.x.06, R. Sundararaj, deposited in the collection of National Forest Insect Collection, Forest Entomology Division, Forest Research Institute, Dehra Dun, India (NFIC # 21861). Paratypes, 3 puparia, data as for holotype, one each deposited in the collection of Division of Entomology, Indian Agricultural Research Institute, New Delhi, India; and Zoological Survey of India, Kolkata, India (2434/H15) and the remaining in the collection of Institute of Wood Science & Technology, Bangalore, India.

Etymology

Named after its host plant family Loganiaceae.

Comments

This species resembles *Dialeurodes sepangensis* Corbett in having thoracic and caudal tracheal pores with distinct teeth, round papilla-like structures throughout dorsum and distinct thoracic and caudal tracheal folds with stipples, but differs from it in having 13 pairs of subdorsal setae, posterior end of vasiform orifice with inner teeth and by the absence of a ring of minute acicular spines.



FIGURES 4–7. *Dialeurodes trilobiata* sp. nov. 4. Puparium; 5. margin with thoracic tracheal furrow; 6. trilobed tubercle; 7. vasiform orifice.

2. *Dialeurodes trilobiata* sp. nov. (Figs. 4–7)

Puparium (Fig. 4): White, with no wax secretion, broadly elliptical, widest across the transverse moulting suture; 1.20–1.44 mm long, 0.90–1.20 mm wide; found singly on the lower surface of leaves. Margin smooth, thoracic and caudal tracheal pores distinct with chitinised rim with inner teeth (Fig. 5). Anterior and posterior marginal setae 16 μm and 20 μm long, respectively.

Dorsum: Submargin not separated from dorsal disc, submarginal striations evident. Subdorsum with broken transverse sutures and granulated ridges. Submedian pockets present on all abdominal segment sutures, submedian depressions faintly discernible in all the abdominal segments. Longitudinal moulting suture reaching margin and transverse moulting suture reaching submedian area. Two pairs of typical round tubercles enclosing three small lobe-like structures (Fig. 6) arranged closely in a triangular fashion one each on submedian area of prothorax and II abdominal segment.

Thoracic tracheal furrows distinct, caudal tracheal furrow, 156 μm long, 30 μm wide at its widest end. Subdorsal/submarginal row of setae absent.

Chaetotaxy: Three pairs of setae-cephalic setae 8μm long, first abdominal setae 4 μm long and eighth abdominal setae cephalolaterad of vasiform orifice 2 μm long. Caudal setae not discernible.

Vasiform orifice (Fig. 7): Subcircular, wider than long, 44–56 μm long, 50–54 μm wide, inner postero-lateral wall of orifice with a comb of teeth and a prominent tooth in the centre; operculum subcircular, slightly expanded at antero-lateral walls, 22–26 μm long, 32–36 μm wide, filling orifice and obscuring lingula.

Venter: Paired ventral abdominal setae 8 μm long, 60 μm apart. Minute setae at the base of pro- meso- and metathoracic legs 5 μm long evident. Thoracic and caudal tracheal folds distinct with stipples. Antennae tip pointed reaching beginning of mesothoracic leg.

Specimens examined

Holotype: puparium, India: Kerala: Munnar, on *Syzygium* sp., 28.iii.07, R. Pushpa, deposited in the collection of National Forest Insect Collection, Forest Entomology Division, Forest Research Institute, Dehra Dun, India (NFIC # 21864). Paratypes, 2 puparia, data as for holotype, one each deposited in the collection of Division of Entomology, Indian Agricultural Research Institute, New Delhi, India and Zoological Survey of India, Kolkata, India (2435/H15); Karnataka: Bangarpet (Markandaya hills), 1 puparium, on *Morinda pubescens*, 9.xii.05, R. Sundararaj; Kerala: Singampara (Palakkad), 1 puparium, on *Cinnamomum malabathrum*, 22.x.06, R. Sundararaj deposited in the collection of Institute of Wood Science & Technology, Bangalore, India.

Etymology

The species name reflects its typical three lobed structure of submedian tubercles.

Comments

This species resembles *Dialeurodes panacis* Corbett, in having two pairs of submedian tubercles one pair each on prothorax and II abdominal segment, but differs from it in the absence of a tubercle on metathorax and microtubercles on all segment sutures.

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Entomology, University of Agricultural Sciences, GKVK, Bangalore for their valuable comments.

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Haemolymph protein polymorphism at pre and post molting stages of the silkworm *Bombyx mori* L.

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ABSTRACT: The total quality and quantity of protein in the haemolymph of insects varies in the course of larval growth and development and the peak concentration is clearly visible at the later larval stages among lepidopterans while, some of the proteins actively participate in different physiological functions during molting period in many insects. The present nding is an attempt utilizing six silkworm races to understand the protein proles through SDS-PAGE during before and after molting. Based on the banding pattern of each races we have demonstrated that appearance and disappearance of specic protein bands occur during molting process. The UPGMA clustering analysis utilizing six races are also herein discussed.
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KEYWORDS: *Bombyx mori*, molting, polymorphism, haemolymph protein, SDS-PAGE

Biochemical characterization through protein proling is very important in the lepidopteran insects, since proteins may change with physiological state, age, race, sex and molting (Wigglesworth, 1964; Chapman, 1998). The *Bombyx mori* belongs to Bombycidae produces a massive amount of haemolymph proteins as primary organic compound which get converted into a delicate twin thread of silk broin, coated by a protective cover of sericin. It is important that, protein variation occurs at every molting stage which increases at pre molting stage popularly denoted as "Peeler stage" and falls at few days after molting popularly denoted as "Soft back stage" (Saravanan *et al.*, 2008). Such type of variations in the proteins of the domesticated silkworm *Bombyx mori* is quite signicant in understa nding the genetic constitution of a race/breed and keeping the above aspects in the mind, the present research work is being undertaken utilizing different geographical races of *Bombyx mori.*

Six silkworm races (Hence forth referred as genotypes) namely two bivoltines NB_4D_2, CSR_2; two multivoltines *npnd*, Hosa Mysore; two mutants Zebra and Knobbed are drawn from the germplasm bank of the Department of Studies in

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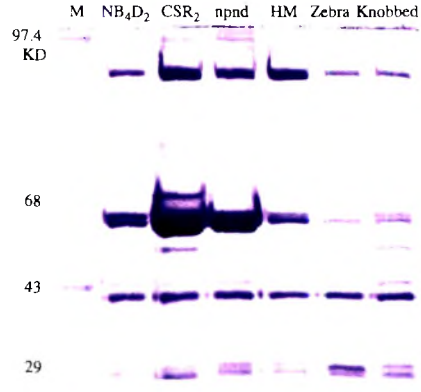


FIG. 1

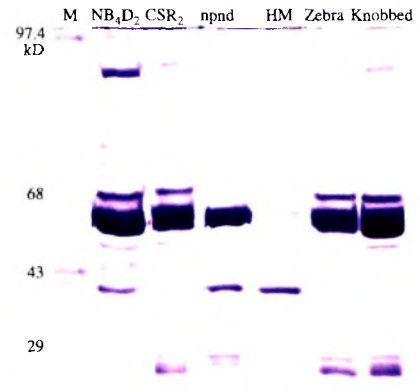


FIG. 2

FIGURES 1 and 2. Electrophoretic protein patterns with marker (M) during pre molting (Fig. 1) and post molting stages (Figs. 2) in six genotypes of the silkworm *Bombyx mori*.

Sericulture Science, University of Mysore and they were reared following the scientic method of rearing (Krishnaswami, 1978). For experimental purpose pre molting (Pr M) IV instar and post molting (Po M) V instar larvae of the above genotypes were selected. Haemolymph protein was extracted from both the stages following the procedure of Tazima (1978) and electrophoresed through 8.25% of SDS polyacrylamide gel electrophoresis.

With reference to literature in regard to insect molting process clearly indicates the consequential haemolymph protein changes which are inuenced by several internal and external factors, viz., neuroendocrine hormones, physiological variations, salinity and temperature (Horodyski and Riddiford, 1989; Chen, 1992; Zhu and Li, 2001). Further, Locke (1998) reported that, cuticular changes in insects during molting are governed by juvenile hormones and molting hormones. Neilsen and Mills (1968) postulated a hypothesis for the appearance and disappearance of protein bands (PB) in the haemolymph of *Periplanata americana*. In regard to haemolymph proteins, Heller (1924) has reported that haemolymph proteins uctuate during the development of *Bombyx mori*. Keeping in view of the above concept, it is clear from our ndings that there is differentially expressed protein banding noticed at both Pr M prole and Po M prole (Figs. 1–3), wherein a maximum of 26 PB were encountered in Zebra genotype at Pr M prole and subsequently, 18 PB were observed at Po M prole of same genotype. On the other hand, the least number of 8 PB were noticed in *npnd* genotype at Po M prole but 20 PB were observed at Pr M prole of same genotype. Moreover, the moderate numbers of differentially expressed PB are 24, 20, 17 and 12 in Knobbed, NB_4D_2, CSR_2 and Hosa Mysore genotypes respectively at Pr M prole with different intensity. Moreover, at Po M prole PB are 18, 18, 17 and 14 in Knobbed, NB $_4D_2$, CSR_2 and Hosa Mysore respectively. In addition, from the investigation the protein fractions could be distinguished into three groups namely I, II and III. All the three

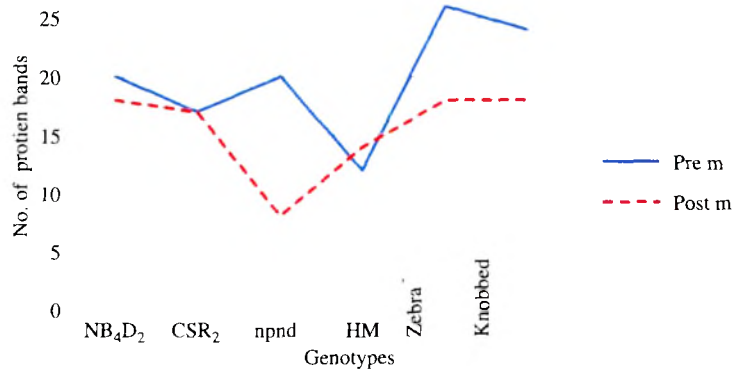


FIGURE 3. Variation of protein bands at pre and post molting stages.

TABLE 1. Characteristic features of six mulberry silkworm genotypes.

Genotypes	Voltinism	Larval pattern	Cocoon colour	Cocoon shape	Origin
NB_4D_2	Bivoltine	Plain	White	Dumb-bell	Indigenous
CSR_2	Bivoltine	Plain	White	Oval	Indigenous
npnd	Multivoltine	Marked	White	Oval	Japanese
Hosa Mysore	Multivoltine	Plain	Dark green	Oval	Indigenous
Zebra	Bivoltine	Marked	White	Dumb-bell	Japanese
Knobbed	Bivoltine	Marked	White	Dumb-bell	Japanese

groups were observed within the range of 29 kDa to 97.4 kDa with a high molecular weight (MW) fractions falling into group I, moderate into group II and III fractions under low MW with their different intensity.

Manifestation of specic bands

Qualitative analysis of haemolymph PB pattern demonstrated appearance and disappearance of specic bands both at Pr M stage and Po M stage (Table 2) with their discretive molecular weight (MW) intensity of 29 kDa, 43 kDa, 68 kDa and 97.4 kDa among the six genotypes.

The observation in our nding is being closely related to the disappearance of PB in Po M stage. In case of NB_4D_2 genotype 20 PB are noticed at Pr M stage whereas, at Po M stage 18 discretive PB was observed in which 2 bands of MW 97.4 kDa and 68 kDa are disappeared. On the other hand, in case of *npnd* genotype totally 20 bands are observed in Pr M stage and after molting a least of 8 bands appeared during Po M prole (four bands each at 97.4 kDa, 68 kDa and 29 kDa). Moreover, in Zebra mutant a highest of totally 26 PB are observed at Pr M prole whereas, at Po M stage 18

TABLE 2. Electrophoretic banding variation in the haemolymph protein proles at pre and post molting stages in six genotypes of the silkworm *Bombyx mori.*

Sl. No.	Genotypes	Molting stages	Indication of Bands
1	NB_4D_2	**Pr M**	++++--++++++++++++--++--++
		Po M	++++++++++++++++++++
2	CSR_2	**Pr M**	++++--++++++--++++--+--++
		Po M	++++-++++++++++++
3	*npnd*	**Pr M**	++++--++++++++++++--++--++
		Po M	++------++++----++
4	Hosa Mysore	**Pr M**	+----++----++++++--++-----++
		Po M	++----++++++++++++
5	Zebra	**Pr M**	++++++++++++++++++++++++++
		Po M	++++++++++++++++++++
6	Knobbed	**Pr M**	++++++++++++++++++++++-+++-++
		Po M	++++++++++++++++++++

Index: + Presence of band.
- Absence of band.

distinct PB are noticed in which 8 PB are disappeared (four bands at 97.4 kDa, two at 68 kDa one each at 43 kDa and 29 kDa). Knobbed genotype showed 24 PB at Pr M and 18 PB at Po M wherein a total 6 discretive PB are disappeared with their MW measured are 2 bands each of 97.4 kDa and 68 kDa and each one of 43 kDa and 29 kDa. But an interesting reverse protein banding pattern was observed in case of Hosa Mysore. It is evident that in Pr M stage 12 differentially expressed PB are observed, while in case of Po M stage totally 14 bands are noticed (two new PB appeared each one of 43 and 29 kDa MW at Po M stage). Infact the reversal trend in the protein banding pattern in this race seems to be quite complex and require further studies. The disappearance of PB may indicate either the non-production or utilization or the degradation of haemolymph proteins to manipulate the molting process in silkworm larval development as reported by Mahesha *et al.* (2000). The hydrolysis of protein might have occurred during the larval period to form amino acids, which in turn, might be utilized to form silk proteins (Beadle and Shaw, 1950).

As evident by the clustering analysis using Unweight Pair Group Mean Anlysis (UPGMA) provided us a different picture altogether (Figs. 4 and 5). With reference to literature it is clear that, the protein variation in different species form the basis to understand the phylogeny and to establish the genetic basis of taxonomic relationship between species and enzyme/protein polymorphism are known to be extremely common and easy to nd in most *Drosophila* species (Hubby and Lewontin, 1966; Lewontin and Hubby, 1966). Further, in plants Ahmad *et al.* (1997) opined that seed protein electrophoresis has also become a useful tool in evolutionary studies to determine species relationship. In the light of the above, the phylogenic relationship

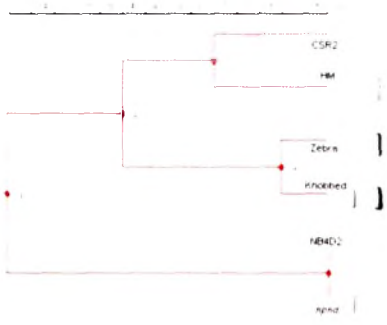


FIG. 4

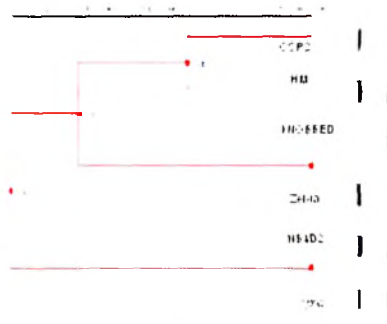


FIG. 5

FIGURES 4 and 5. Dendrograms showing phylogenic relationship between each six silkworm genotypes at pre and post molting stages.

and genetic distance derived from protein proles through dendrogram representation (UPGMA) has showed the geographical, taxonomical clustering relationship and genetic distance between the six genotypes at Pr M and Po M proles.

The UPGMA dendrogram showed that, genotypes are constantly falling into their respective clusters both in Pr M and Po M stages. In case of Pr M stage, the CSR_2 and Hosa Mysore are falling in one cluster (Both produce oval type of cocoons), Zebra and Knobbed genotypes are falling into second cluster (Mutant genotypes producing dumb-bell type of cocoons) and genotypes of NB_4D_2 (Dumb-bell type of cocoons) and *npnd* (Oval type of cocoons) are falling into the third ultimatum cluster and the genetic distances between three clusters are 0.72%, 0.95% and 0.00% respectively. In addition to this, the Po M prole dendrogram revealed, similar relationship of afore explained dendrogram (Pr M) between the six genotypes even though they belongs to same cluster but genotypes of second cluster exchanges between them but remain in same cluster with genetic distances 0.64%, 0.25% and 0.00% respectively.

The present investigation of the authors indicates two important aspects in regard to molting in silkworms. Though there exists variations in the protein pattern at pre and post molting stage proles yet, such supportin g results are not clearly evident through UPGMA from both the proles. The present ndings of the authors thus adds to the knowledge of genetic difference between silkworms which is quite useful in silkworm genetics and breeding.

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Comparative studies on the diversity of butterflies between coastal and arid zones

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ABSTRACT: Butterflies are biological indicators of the habitats. They are very sensitive to their environment, their very presence or absence indicates the health of the environment. Though there are reports of butterfly diversity in various parts of our country, hardly there are any reports on comparative studies on these organisms. Hence, comparative studies on diversity of butterflies in coastal and arid zone were conducted. The coastal area of Goa was compared with arid zone Bagalkot (Karnataka State), for the diversity of butterflies. During the study period, a total of 80 species of butterflies, of which 56 species of butterflies were from arid zone Bagalkot (Karnataka) and 50 species were recorded from coastal region Goa. Among these, members belonging to the family Nymphalidae, were the most common. The maximum diversity and abundance was observed in the arid region, with around 26 species common to both. 30 different species restricted only to arid area and 24 different species to the coastal area.. Result showed that, the populations differed in variability and responses to endogenous and exogenous factors. © 2011 Association for Advancement of Entomology

KEYWORDS: Arid zone, Bagalkot, butterfly species, coastal area, Goa, habitat, diversity

INTRODUCTION

Among insects, butterflies are the most studied group taxonomically, which have received a reasonable amount of attention throughout the world (Ghazoul, 2002). Many of butterfly species are strictly seasonal and prefer only a particular set of habitat (Kunte, 1997) and they are good indicators in terms of anthropogenic disturbance and habitat quality (Kocher and Williams, 2000). The topics such as butterfly community assemblage and factors influencing it, have been of interest to ecologists and conservationists. Since the turn of the 19th century, many workers (Bingham, 1905, 1907; Williams, 1927) have recorded butterfly species in south India. In 1987, Larsen made a detailed survey of butterflies of Nilgiri Mountains and recorded nearly 300 species including endemic ones. Many researchers have

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contributed to our understanding of butterfly abundance and diversity (Gascon *et al.*, 1999; Kunte *et al.*, 1999; Rickets *et al.*, 2001; Pai, 2002; Arun and Azeez, 2003; Eswaran and Pramod, 2005; Xavier, 2006; Pramod Kumar *et al.*, 2007; Krishnakumar *et al.*, 2008) and on habitat association, effect of disturbance and area clearance (Soubadra and Priya, 2001; Sreekumar and Balakrishnan, 2001; Dolia *et al.*, 2008), seasonal abundance and migration patterns (Kunte, 1997, 2000; Arun, 2002; Kunte, 2005; Padhye *et al.*, 2006) and on conservation (Mathew and Binoy, 2002; Mathew and Anto, 2007; Kunte, 2008; Ramesh *et al.*, 2010) from Western Ghats.

However, almost no attention has been paid to understand the butterfly diversity, density and community composition in arid zones. Further, a comparative study between coastal areas and arid plains, to understand the butterfly diversity and distribution pattern is unheard. Hence, to fill these lacunae, an attempt was made to study and compare the butterfly diversity and distribution pattern in coastal regions of Goa with that of arid zone of Bagalkot (Karnataka).

MATERIAL AND METHODS

The present study started with a view to examine the different species of butterflies across two different habitats namely coastal Goa and arid zone Bagalkot.

Study site: Goa, the land of sun, sand and sea, lies between 15°48′00″N and 14°53′54″N and 74°20′13″E and 74°40′33″E, though has a limited land area of about 3701 Km^2 is warm humid typical coastal weather. Bagalkot district, Karnataka State (160° 12′N 750° 45′E), is basically an arid zone and has warm and dry weather. Both places, exhibit a diversity of plant species, which are congenial for the butterflies.

Collection by using net was the primary method of collection. In addition to sweeping, baiting and collecting and rearing of immature stages were also used. In Goa, the coastal areas, 3–5 km from coast line was surveyed. While in Bagalkot, almost all places, in district were covered. In both the cases, opportunistic survey method was employed.

RESULTS AND DISCUSSION

During the study period, a total of 80 species of butterflies, of which 56 species of butterflies were from arid zone Bagalkot (Karnataka) and 50 species were recorded from coastal region Goa (Table 1). Among these, members belonging to the family Nymphalidae, were the most common. A similar pattern of predominance of *Nymphalidae* was also reported by different researchers (Kunte, 1997; Kunte *et al.*, 1999; Eswaran and Pramod, 2005; Dolia *et al.*, 2008; Krishnakumar *et al.*, 2008; Soubadra and Priya, 2001; Pramod Kumar *et al.*, 2007; Padhye *et al.*, 2006) from Western Ghats.

Fig. 1, exhibits the Graph of Families of Butterflies V/s number of different species of Butterflies, wherein members of Nymphalidae show maximum number in both arid and coastal regions under study.

Fig. 2 (Graph) shows the number of different families of butterflies V/s number of same species of Butterflies found in these two regions. The graph indicates that,

TABLE 1. Distribution pattern of Butterflies in the arid and coastal regions

Sr. No.	Common name	Scientific name	Arid Area	Coastal Area (Pai, 2002)
1	Blue Mormon	*Papilio polymnestor* (Cramer)	✓	✓
2.	Common bottle blue	*Graphium sarpedon* (Linnaeus) (=*Zetides sarpedon* (Linnaeus))	✓	-
3.	Common Mormon	*Papilio polytes polytes* (Linnaeus)	✓	✓
4.	Common Mormon	*Papilio polytus Romulus* (Cramer)	✓	-
5.	Common Mormon	*Papilio polytus stichius* (Hubner)	✓	-
6	Common Rose	*Pachliopta aristolochiae* (Fabricius) (=*Tros aristolochiae* (Fabricius))	✓	✓
7	Crimson Rose	*Pachliopta hector* (Linnaeus) (=*Tros hector* (Linnaeus))	✓	✓
8.	Lime Butterfly	*Papilio demoleus* (Linnaeus)	✓	-
9.	Red Helen	*Princeps helenus* (Linnaeus)	✓	
10	Southern Birdwing	*Troides minos* (Cramer) (=*T. helena*)	-	✓
11	Tailed Jay	*Graphium agamemnon* (Linnaeus) (=*Zetides agamemnon* (innaeus))	✓	✓
12.	Capers	*Catopsilia pyranthe* (Linnaeus)	✓	-
13.	Common/Lemon Emigrant	*Catopsilia crocale* (Cramer)	✓	✓
14.	Common Albatross	*Appias albino* (Felder)	✓	-
15.	Common Gull	*Cepora nerissa* (Fabricius) (=*Huphina nerissa* (Fabricius))	✓	✓
16.	Common Jezebel	*Delias eucharis* (Drury)	✓	✓
17.	Common Wanderer	*Pareronia valeria* (Fabricius)	✓	✓
18.	Crimson tip	*Colotis danae* (Frabricius)	✓	-
19.	Great orange tip	*Hebomoia glaucipe* (Linnaeus)	✓	-
20	Mottled Emigrant	*Catopsilia pyranthe* (Linnaeus)	✓	✓
21.	Painted saw tooth	*Prioneris sita* (C.Felder)	✓	-

TABLE 1. Contd . . .

22.	Pioneer white	*Belenois aurota* (Fabricius)	✓	-
23.	Plain orange tip	Colotis eucharis (Fabricius)	✓	-
24.	Psyche	*Leptosia nina* (Fabricius)	✓	✓
25.	Small / little orange tip	*Colotis etrida* (Boisdvual)	✓	-
26.	Small Grass Yellow	*Eurema hecabe simulate* (Cramer) (=Terias hecabe (Linneaus))	✓	✓
27.	Small salmon Arab	*Colotis amata* (Fabricius)	✓	-
28.	White orange tip	*Ixias Marianne* (Fabricius)	✓	-
29.	Yellow Orange tip	*Ixias pyrene* (Cramer)	✓	-
30.	Baron	Euthalia aconthea (Hewitson) (=*Euthalia garuda* (Moore))	✓	✓
31.	Black Prince	*Rohana parisatis* (Moore)	-	✓
32	Black Rajah	*Charaxes solon solon* (Fabricius.) (=*Charaxes fabius)* (Fabricius.)	-	✓
33.	Blue pansy	*Junonia orithya* (Linnaeus) (=Precis orithya)	✓	-
34.	Blue Tiger	*Tirumala limniace exoticus* (Butler) *(=Danaus limniace leopardus)*	-	✓
35.	Bush brown	*Mycalesis subdita* (Moore)	✓	✓
36.	Chocolate Pansy	*Junonia iphita* (Cramer) (=*Precis iphita*)	✓	✓
37.	Commander	*Moduza procris undifragus* (Cramer) *(=Limenitis procris)*	-	✓
38.	Common Castor	*Ariadne merione* (Cramer) (=*Ergolis merione* (Cramer))	✓	✓
39.	Common Evening Brown	*Melanitis leda* (Linnaeus) (=*Melanitis leda ismene* (Cramer))	✓	✓
40.	Common Fivering	*Ypthima baldus* (Fabricius)	✓	-
41.	Common Indian Crow	*Euploea core* (Cramer)	✓	✓
42.	Common Leopard	*Phalanta phalantha* (Drury) *(=Atella phalantha)*	-	✓
43.	Common Palmfly	*Elymnias hypermnestra caudate* (Drury)	-	✓

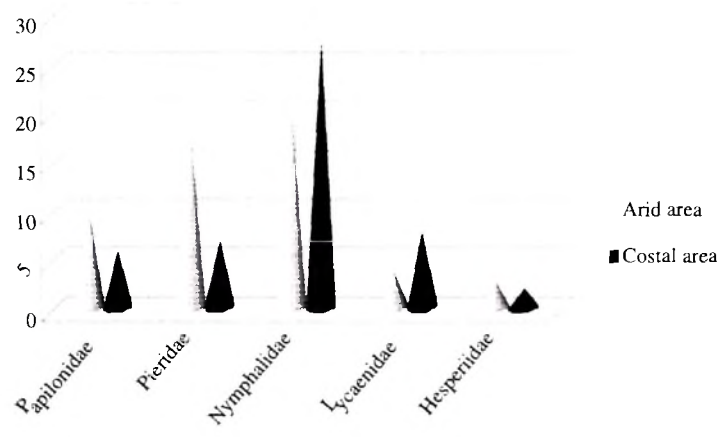
TABLE 1. Contd . . .

44.	Common Sergeant	*Athyma perius* (Linnaeus) (=*Pantoporia perius*)	-	✓
45.	Danaid Eggfly	*Hypolimnas misippus* (Linnaeus) (=*Diadema misippus*)	✓	✓
46.	Dark Blue Tiger	*Tirumala septentrionis dravidarum* (Butler) (=*Danaus melissa*)	-	✓
47.	Dark band Bushbrown	*Mycalesis mineus* (Linnaeus)	✓	-
48.	Glassy Blue Tiger	*Parantica aglea* (Stoll) (=*Danaus aglea* (Cramer))	✓	✓
49	Great Eggfly	*Hypolimnas bolina jacintha* (=*Hypolimnas bolina* Linnaeus)	✓	✓
50.	Great evening brown	*Melanitis zitenius* (Herbst)	✓	-
51	Grey Count	*Tanaecia lepidea mixana* (Frushstorfe) (=*Euthalia lepidea* Butler)	-	✓
52	Grey Pansy	*Precis atlites* (Johanssen)	-	✓
53.	Joker	*Byblia ilithyia* (Drury)	✓	-
54	Lemon Pansy	*Junonia lemonias* (Linnaeus) (=*Precis lemonias*)	✓	✓
55.	Map Butterfly/ common Map	*Cyrestis thyodamas indica* (Evans)	✓	✓
56.	Painted Lady	*Vanessa cardui* (Linnaeus)	-	✓
57.	Peacock Pansy	*Junonia almana* (Linnaeus) (=*Precis almana* (Linnaeus))	✓	✓
58.	Plain Tiger	*Danaus chrysippus* (Linnaeus) (=*Danais chrysippus* (Linnaeus))	✓	✓
59.	Striped Tiger	*Danaus genutia* (Cramer) (=*Danais plexippus* (Linnaeus))	✓	✓
60.	Tamil Yeoman	*Cirrochroa thais thais* (Fabricius)	-	✓
61.	Tawny coster	*Acrae terpsicore* (Linnaeus)	✓	-
62	Yellow Jack Sailor	*Lessipa viraja viraja (moore)*	-	✓
63.	Yellow Pansy	*Junonia hierta* (Fabricius)	✓	-

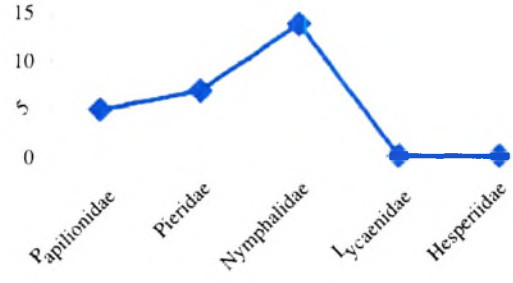
TABLE 1. Contd . . .

64.	Branded Blue pierrot	*Discolampa ethion* (Westwood) (=Castalius ethion)	✓	-
65.	Common Cerulean	*Jamides celeno aelianus* (Fabricius) (=*Jamides celeno celeno* (Cramer))	-	✓
66.	Common Gem	*Poritia hewitsoni hewitsoni* (Moore)	-	✓
67	Common Pierrot	*Castalius rosimon rosimon* (Fabricius)	-	✓
68.	Common silverline	*Castalius rosimon rosimon* (Fabricius)	✓	-
69.	Cornelian	*Deudorix epijarbus amatius* (Moore)	-	✓
70.	Forget me not	*Catochrysops Strabo* (Fabricius)	✓	-
71.	Gram Blue	*Euchrysops enejus* (Fabricius)	✓	-
72.	Monkey Puzzle	*Rathinda amor* (Fabricius)	-	✓
73.	Red Pierrot	*Talicada nyseus nyseus* (Guirin-Menevile)	-	✓
74.	White banded Royal	*Ancema cotys (Hawitson) (=Pratapa cotys)*	-	✓
75.	Yamfly	*Loxura atymnus atymnus* (Cramer)	-	✓
76.	Dark banded swift	*Pelopidas agna* (Moore)	✓	-
77.	Indian skipper/ Indian Grizzled	*Spialoa galba* (Fabricius)	✓	-
78.	Malabar Flat	*Celaenorrhinus ambareesa* (Moore)	-	✓
79.	Multi Spotted Flat	*Celaenorrhinus pulomaya pulomaya* (Mielke)	✓	-
80.	Small branded Swift	*Pelopidas mathias (Fabricius)*	✓	-

maximum number of species of butterflies encountered in the present studies was from Nymphalidae and the Hesperiedae represented by minimum number of butterflies. Is also interesting to note that, some species of butterflies showed specificity or restriction to particular habitat for eg., *Graphium sarpedon* (Linnaeus), *Papilio polytes romulus* (Cramer). *Papilio polytes stichius* (Hubner). *Papilio demoleus* (Linnaeus).



FIGURES 1. Graph of Families of Butteries V/s number of different species of Butteries.



FIGURES 2. Graph of Different families of butteries V/s number of same species of butteries found in these two regions.

Princeps helenus (Linnaeus), belonging to family papilionidae were noticed only in the arid area/region. Similarly *Troides minos* (Carmer), belonging only to coastal region.

The buttery species such as *Catopsilia pyranthe* (Linnaeus), *Appias albina* (C. Felder), *Colotis danae* (Fabricius), *Hebomoia glaucippe* (Linnaeus), *Prioneris sita* (C. Felder), *Belenois aurota* (Fabricius), *Colotis eucharis* (Fabricius), *Colotis etrida* (Boisduval), *Colotis amata* (Fabricius), *Ixias marianne* (Cramer), *Ixias pyrene* (Linnaeus) belonging to family Pieridae and showed the specicity only towards arid region and none from coastal area.

Species of the family Nymphalidae such as *Junonia orithya* (Linnaeus), *Ypthima baldus* (Fabricius), *Mycalesis mineus* (Linnaeus), *Melanitis zitenius* (Herbst), *Byblia ilithyia* (Drury), *Acreae terpsicore* (Linnaeus), *Junonia hierta* (Fabricius) restricted only to arid region and *Rohana parisatis* (Moore), *Charaxes solon solon* (Fabricius), *Tirumala limniace exoticus* (Butler), *Moduza procris undifragus* (Cramer), *Pha-*

lanta phalantha (Drury), *Elymnias hypermnestra caudata* (Drury), *Athyma perius* (Linnaeus), *Tirumala septentrionis dravidarum* (Butler), *Tanaecia lepidea miyana* (Butler), *Precis atlites* (Johanssen), *Vanessa cardui* (Linnaeus), *Cirrochroa thais thais* (Fabricius), were found only in coastal area. Species belonging to family Lycaenidae *Discolampa ethion* (Westwood), *Spindasis vulcanus vulcanus* (Fabricius), *Catochrysops strabo* (Fabricius), *Euchrysops cnejus* (Fabricius), found only in the arid region and *Jamides celeno aelianus* (Fabricius), *Poritia hewitsoni hewitsoni* (Moore), *Castalius rosimon rosimon* (Fabricius), *Deudorix epijarbus amatius* (Moore), *Rathinda amor* (Fabricius), *Talicada nyseus nyseus* (Guerin-Meneville), *Ancema cotys* (Hawitson), *Loxura atymnus atymnus* (Fruh), were found specic only to the coastal region. *Pelopidas agna* (Moore), *Spialia galba* (Fabricius), *Pelopidas mathias* (Fabricius), belonging to family Hesperiidae were specic only to the arid region and *Celaenorrhinus ambareesa* (Moore), *Celaenorrhinus pulomaya pulomaya* (Mielke), species of butteries were specic to the coastal region.

Apart from the above, some species of butteries such as *Papilio polymnestor* (Cramer), *Papilio polytes polytes* (Linnaeus), *Pachliopta aristolochiae* (Fabricius), *Pachliopta hector* (Linnaeus), *Graphium agamemnon* (Linnaeus), *Catopsilia crocale* (Cramer), *Cepora nerissa* (Fabricius), *Delias eucharis* (Drury), *Pareronia valeria* (Fabricius), *Catopsilia pyranthe* (Linnaeus), *Leptosia nina* (Fabricius), *Eurema brigitta* (Cramer), *Euthalia aconthea* (Hewitson), *Mycalesis subdita* (Moore), *Junonia iphita* (Cramer), *Ariadne merione* (Cramer) , *Melanitis leda* (Linnaeus), *Euploea core* (Cramer), *Hypolimnas misippus* (Linnaeus), *Parantica aglea* (Stoll), *Hypolimnas bolina jacintha* (Linnaeus), *Junonia lemonias* (Linnaeus), *Cyrestis thyodamas indica* (Evans), *Junonia almana* (Linnaeus), *Danaus genutia* (Cramer) *and Danaus chrysippus* (Linnaeus) were found common to both habitats.

Thus the studies show that, whether it is coastal or arid zone, butteries do show diversity, probably depending upon various aspects such as agro-climatic condition, ora, availability of food etc., There may be several common species of butteries found in both coastal and arid zone, but there too exists some specic species of butteries for coastal and arid zones. Th ese species can be considered as indicator species of a specic agro-climatic condition and further in case any change occurs in the climatic conditions, these butteries can be used as indicator species to indicate such climatic changes.

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Effect of starvation on glutamate-oxaloacetate transaminase activity in *Bombyx mori* L.

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ABSTRACT: An attempt was made to study the effect of starvation on the activity of glutamate-oxaloacetate transaminase (GOT) in midgut, haemolymph and silk gland of Polyvoltine (PV), Bivoltine (BV) and Cross breed (CB) varieties of silkworm, *Bombyx mori* during fifth instar larval development. The results show that in the midgut, the enzyme activity increased in all the three varieties with both 2 h and 4 h starvation. In the haemolymph, the enzyme activity increased in PV and BV and decreased in CB. In silk gland, the activity of GOT decreased in PV and CB and increased in BV. The results are discussed in relation to the degree of digestion, absorption and interconversion of specific amino acid for silk protein synthesis during fifth larval stage. © 2011 Association for Advancement of Entomology

KEYWORDS: *Bombyx mori*, fifth instar, starvation, transaminase

INTRODUCTION

The basic process of morphogenesis during development of an organism is the cell differentiation which is biochemically indicated through the metabolism of proteins. The metabolism of proteins largely depends upon the free amino acid pool, the intermediary pathways of individual amino acids as well as interconversion of amino acids through the activities of specific transaminases. The overall metabolic processes, in addition to providing energy, also provide required precursors for the formation of major biomolecules like proteins, carbohydrates, lipids, etc., in the body of an organism (Meister, 1965). In silkworm, the activity of transaminases has been demonstrated in various tissues (Gowda and Ramaiah, 1975; Klunova *et al.*, 1976; Irving *et al.*, 1979a,b). Hirayama and Nakamura (2002) have observed that the changes in glutamine synthetase activity in silkworm haemolymph was relatively small throughout the larval development. Ravikumar and Sarangi (2005) have reported that the activities of AAT and GOT in different tissues of silkworm leads to the conversion of specific amino acids required directly or indirectly for the synthesis of silk proteins during fifth instar larval development. In silkworms, starvation results in

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decrease in the concentration of carbohydrates (Shamala and Sarangi, 2001), protein content and midgut protease activity (Roopa *et al.*, 2001). The concentrations of Cys, His, Arg, and Gly have been shown to increase with 48 h starvation in the haemolymph of *Philosamia cynthia* (Bosquet, 1977). However, information on the effect of starvation on the activities of specific transaminases are scanty. Keeping this in view, an attempt was made to study the effect of starvation on the activity of GOT to analyse the rate of interconversion of amino acids.

MATERIALS AND METHODS

Animal:

Bivoltine (BV) (CSR_2), Polyvoltine (PV) (Pure Mysore) races of *Bombyx mori* and their hybrid (CB) (PM♀ × CSR2♂) were selected for the study and reared under standard laboratory conditions according to the method of Krishnaswami (1978). For the experiment, fifth instar larvae were used from first day to the spinning stage at 24 h intervals. The out of moult fifth instar larvae were segregated in to two separate batches of 100 worms each. The Batch I and Batch II were subjected to starvation for 2 h and 4 h, respectively. The starvation was effected just after bed cleaning every day before resumption of first feeding and maintaining the normal feeding thereafter for the day throughout the fifth instar period. The worms from the mass rearing which followed the normal feed schedule, served as the control. Trial experiments were conducted to determine the durations of starvation.

Tissue preparation:

The silkworms were pre-chilled (Boctor and Salem, 1973) and then haemolymph was collected by cutting open the caudal horn in a pre-chilled haemolymph tube containing a pinch of thiourea (Wyatt and Pan, 1978). The haemolymph was diluted with ice-cold phosphate buffer, pH 7.4, centrifuged at 3000 rpm and the supernatant was used for the enzyme assay. The midgut and silk gland were excised from the same larvae in ice-cold distilled water. A 25% (w/v) homogenate of the tissue was prepared in ice-cold phosphate buffer, pH 7.4, centrifuged at 3000 rpm and the supernatant was used as the enzyme source.

Assay of AAT:

The activity of glutamate-oxaloacetate transaminase (GOT) was assayed according to the method of Reitman and Frankel (1956) using sodium pyruvate as standard. 100 μl of enzyme was incubated with 500 μl of 0.1 M phosphate buffer, pH 7.4 containing 1 mM 2-oxoglutarate and 100 mM aspartate for 30 min at 37°C. The reaction was terminated by adding 500 μl of chromogen solution containing 2,4, dinitrophenyl hydrazine and 0.1N HCl. The mixture was allowed to stand for 20 min at room temperature. Five ml of 0.4 N NaOH was added and the absorbancy was measured after 5 min at 546 nm using Genova UV/Visible spectrophotometer. The data were

analyzed statistically (Panse and Sukhatme, 1989) and the mean values along with standard deviation are presented in the results.

RESULTS

The activity of glutamate-oxaloacetate transaminase (GOT) in midgut, haemolymph and silk gland of all the three varieties of silkworms subjected to different durations of starvation during larval period are presented in the figures.

The GOT activity in midgut of PV increased throughout fifth instar development to reach the peak activity of 72.533 and 72.419 m mole/min/mg on the last day in 2 h and 4 h starvation, respectively (Fig. 1a). Interestingly, in BV, no considerable difference was observed in both the starved batches compared to control (Fig. 1b). In CB, 4 h starvation showed the highest GOT activity of 93.485 m mole/min/mg on the fifth day whereas, 2 h starvation showed no significant difference compared to control (Fig. 1c).

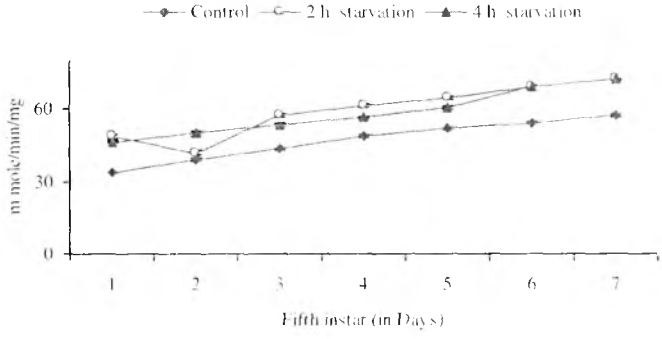
In haemolymph of PV, the GOT activity was found to increase in both the durations of starvation attaining the peak value of 51.468 and 45.774 m mole/min/ml on the last day in 2 h and on sixth day in 4 h starvation, respectively (Fig. 2a). In BV, 4 h starved batch showed the highest GOT activity of 83.806 m mole/min/ml on the third day which decreased thereafter, with no much difference in 2 h starvation compared to control (Fig 2b). But in CB, both the starved batches showed a decrease in GOT activity being the lowest of 7.971 and 10.305 on the second day in 2 h and 4 h starvation respectively (Fig. 2c).

In silk gland of PV, the GOT activity significantly decreased under starvation compared to control (Fig. 3a). In BV, the GOT activity increased significantly throughout the fifth instar under both the durations of starvation (Fig 3b). In CB, the activity of GOT showed a decrease under both the durations of starvation showing the lowest activity of 53.233 on the first day in 4 h starvation (Fig 3c).

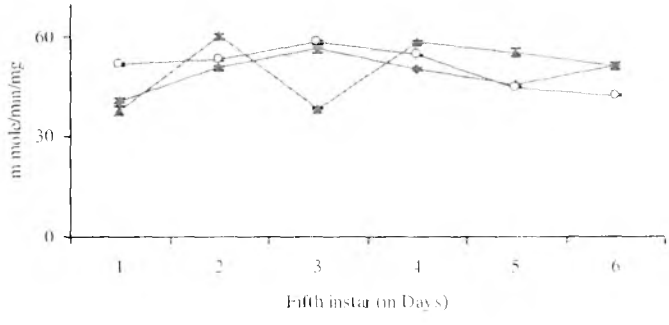
DISCUSSION

In insects, amino acid pool in the body is determined by the rate of digestion of proteins and uptake of amino acids. In addition to the dietary supply, interconversion of amino acids contribute maximally for the build up of amino acid pool in the body. The interconversion mechanism catalyzed by various transaminases is responsible for the synthesis of specific amino acids which are required in large quantities during synthesis of silk proteins in silkworms. In the present study the GOT activity in the midgut increased in both the starved batches of all the silkworm varieties studied compared to control. The midgut of insects has higher concentration of glutamate (Murdock and Koidl, 1972) due to the presence of high affinity membrane transporters for glutamate uptake (Donly *et al.*, 1997; Giordana *et al.*, 1998). Thus the increase in GOT activity under starvation can be directly correlated with the higher uptake of glutamate by the midgut. Further, it can be suggested that the concentration of glutamate is maintained by the chemical nature of midgut cells rather than directly depending upon the presence or absence of food.

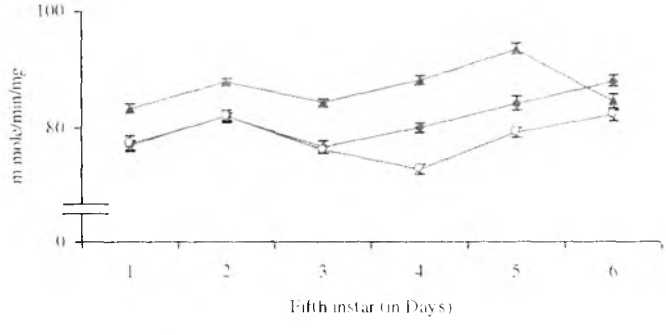
(a) Polyvoltine



(b) Bivoltine

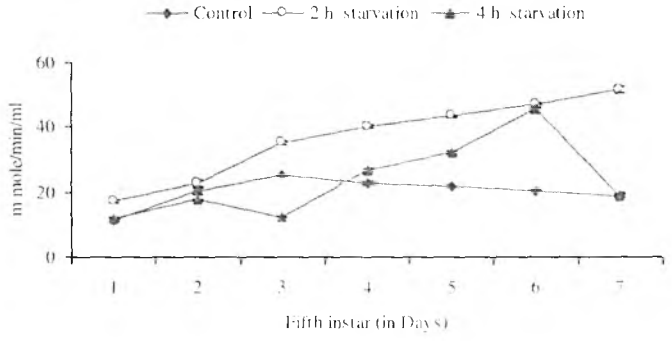


(c) Cross breed

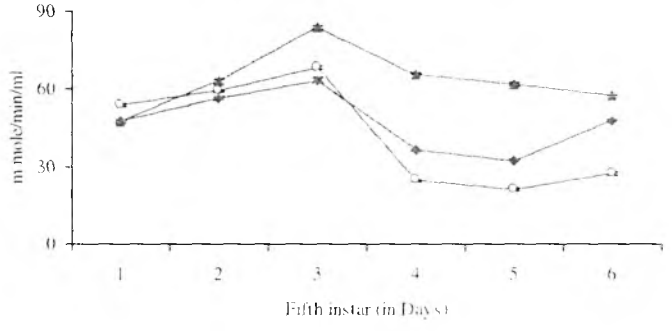


FIGURES 1. Effect of starvation on the activity of GOT in midgut of different varieties of the silkworm, *B. mori*. (a). Polyvoltine; (b). Bivoltine; (c). Cross breed.

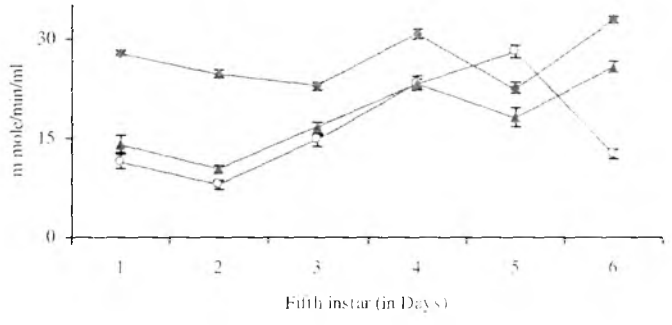
(a) Polyvoltine



(b) Bivoltine

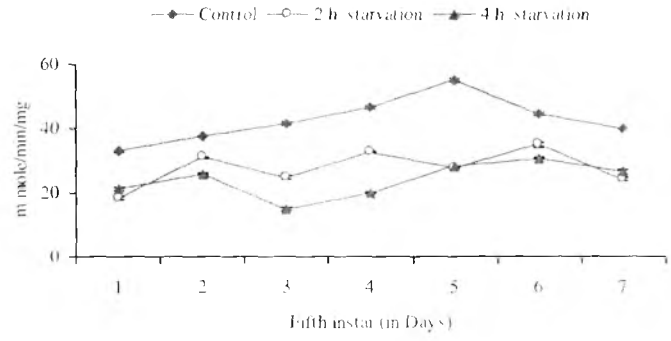


(c) Cross breed

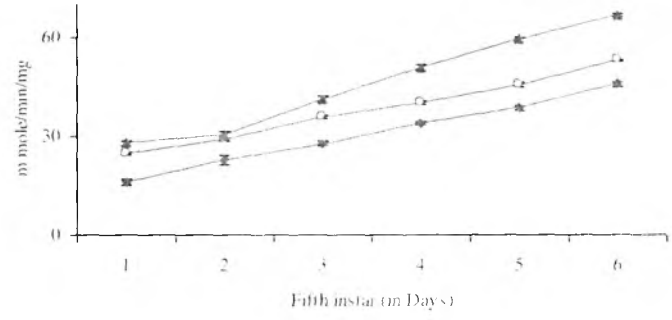


FIGURES 2. Effect of starvation on the activity of GOT in haemolymph of different varieties of the silkworm, *B. mori*. (a). Polyvoltine; (b). Bivoltine; (c). Cross breed.

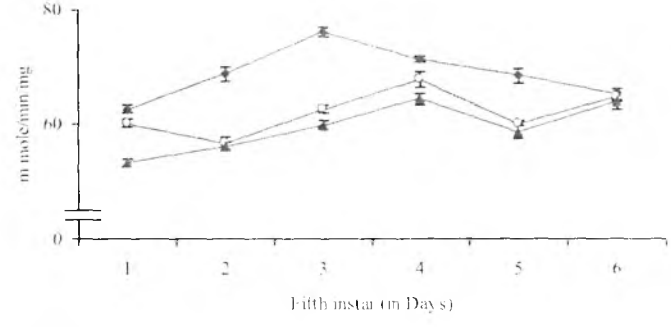
(a) Polyvoltine



(b) Bivoltine



(c) Cross breed



FIGURES 3. Effect of starvation on the activity of GOT in silk gland of different varieties of the silkworm, *B. mori*. (a). Polyvoltine; (b). Bivoltine; (c). Cross breed.

The GOT activity in the haemolymph of both the pure races (PV and BV) increased under both the starvation (2 h and 4 h) experiments, but the CB showed a decreased enzyme activity compared to control. The increased GOT activity in PV and BV is perhaps due to the availability of substrates being released from the fat body. This is in accordance with the earlier reports of Levenbook (1962) who has observed that the absence of glutamate in the haemolymph is actively compensated by the release of glutamate from the fat body. Hence starvation does not have a significant effect on the GOT activity, which shows an increase even in the absence of food. The decrease in GOT activity in CB may be due to the fact that there might exist a varietal difference in the release of glutamate from the fat body.

In silk gland, both the starved batches showed an increased GOT activity in BV, whereas the enzyme activity decreased in PV and CB compared to control. The higher GOT activity under starvation shows clearly that the BV with a capacity to produce more silk perhaps utilizes glutamate in the silk gland being transported from other tissues like midgut and/or haemolymph, to suffice the increased silk protein synthesis. However, in PV and CB, even though the haemolymph and midgut show higher GOT activity, the silk gland fails to cope with those tissues. Thus it is evident from the present results that metabolic activity in the silk gland varies significantly in different silkworm varieties depending upon their capacity to produce the quantity of silk proteins ultimately resulting in the differential silk output in different silkworm varieties.

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Developmental biology and life table studies of *Gyranusoidea indica* Shafee, Alam and Agarwal (Hymenoptera: Encyrtidae) a parasitoid of *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae)

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ABSTRACT: The developmental biology and life table parameters for *G. indica* on *M. hirsutus* were determined. The net reproductive rate, R_0 was 1378.25 females/female/generation, the innate capacity for increase, r_m was 0.3289 eggs/female/day. The generation time was 21.98 days and doubling time was 2.10 days. *G. indica* is synovigenic and the third instar female *M. hirsutus*was the optimum stage for production of a large female population. Male and female longevity were 16.08 ± 1.75 and 28.65 ± 1.35 days respectively. Results indicate that G. *indica's* potential to reduce *M. hirsutus* infestation is more efficient than if used with *A. kamali*. The results presented are essential for mass production, timing of field releases of *G. indica* and development of an effective biological programme for *M. hirsutus*. © 2011 Association for Advancement of Entomology

KEYWORDS: *Gyranusoidea indica*, *Maconellicoccus hirsutus*, fecundity life table

INTRODUCTION

The Encyrtidae is one of the most important chalcidoid families used in classical biological control programmes internationally. Included in this family is *Gyranusoidea* spp. which is a cosmopolitan genus with over 20 described species (Noyes and Hayat, 1994). *Gyranusoidea tebygi* Noyes (Hymenoptera: Encyrtidae) has been successfully used against the mango mealybug *Rastrococcus invadens* Williams (Hemiptera: Pseudoccidae) in Africa in the 1980's and *Gyranusoidea litura* Prinsloo (Hymenoptera: Encyrtidae) was used to control *Pseudococcus longispinus* (Targioni-Torzetti) (Hemiptera: Pseudoccidae) in greenhouses in Belgium, France, Germany

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and the Netherlands in the 1990's. Michaud and Evans (2000) described *G. indica* as a primary parasitoid of *M. hirsutus* and *Nipaecoccus viridis* (Newstead) (Hemiptera: Pseudococcidae) which was described from India. It was recovered by Goolsby *et al.* (2002) during their exploration for natural enemies of *M. hirsutus* in Australia and was reported to be of the same D2 sequencing as *G. indica* held in rearing colonies in California. *Gyranusoidea indica* is morphologically close to *Gyranusoidea hecale* Noyes & Hayat (Hymenoptera: Encyrtidae) and to *G. tebygi* (Noyes and Hayat, 1994). The life cycle of this parasitoid varies with temperature and host stage attacked and its laboratory method of production appears to be the same as with *A. kamali*.

A common criticism of biological control is the lack of quantitative evaluation studies after natural enemies have been released (Luck *et al.*, 1988). Life tables provide a powerful technique for such evaluations because they provide a detailed description of age specific mortality of individuals in the population (Hoddle *et al.*, 1996; Southwood and Henderson, 2000). Fecundity life tables are also an important component in estimating the vital statistics of an insect population (La Rossa *et al.*, 2002) including the net reproductive rate (R_0), the intrinsic rate of increase (r_m), generation time (T), doubling time (DT) and the infinite rate of increase (λ). There is a dearth of basic information on the biology of *G. indica* under both field and laboratory and consequently we present here for the first time a fecundity life table for this important parasitoid on its host *M. hirsutus*. This life table will enable predictions about the growth or decline of populations to be made.

MATERIALS AND METHODS

To successfully rear sufficient numbers of *G. indica* for use in these studies it was necessary to produce adequate quantities of sprouted potatoes (*Solanum tuberosum*), a suitable host for *M. hirsutus* as well as large quantities of *M. hirsutus*, a suitable host for this parasitoid.

Production of sprouted *Solanum tuberosum*

Solanum tuberosum used for human consumption were obtained from the open market and washed with a 5% disinfecting solution of sodium hypochlorite before being air dried. Dried potatoes were placed in a dark room at 20°C and 70%RH in order to promote longitudinal growth of the sprouts and to limit the production of solanine which may be toxic to *M. hirsutus*.

Rearing of *M. hirsutus*

To optimize production of *M. hirsutus*, a cyclical rearing scheme was devised. Every week 40 sprouted potatoes were placed in a paper towel lined plastic tray and this placed in an oviposition cage (60 cm × 60 cm × 60 cm) covered with white organza mesh (40 mesh/cm^2). Each potato was initially infested with a *M. hirsutus* ovisac containing approximately 200–300 eggs. Three weeks post infection there were mainly 3rd instar *M. hirsutus* on each potato. The cultures were maintained in the dark room at 28 ± 2°C and 60 ± 10% RH.

Rearing of *G. indica*

One hundred female *G. indica* were released weekly into the oviposition cage containing 20 potatoes infested with predominately 3rd instar *M. hirsutus*. Mummified mealybugs were collected with a fine tip camel hair brush (size 0) from Day 20 and each placed in an individual round bottom transparent plastic vial (2.5 cm diameter × 9.5 cm with a 0.75 cm diameter hole in the centre of the screw cap covered with organza mesh) until eclosion. On eclosion, a pair of male and female *G. indica* was placed in a similar plastic vial and left to mate for 24 hours. These insect cultures were maintained in the laboratory at 28 ± 2°C and 60 ± 10% RH under a photoperiod of 8L:16D.

Developmental biology of *G. indica*

A large population of ovipositing adult female *G. indica* was introduced into an oviposition cage previously described and containing a tray of 20 sprouted potatoes infected with 3rd instar *M. hirsutus* and left for 24 hours before removing all parasitoids. Small drops of honey were placed on the organza mesh as a food source for the parasitoids. Ten mealybugs were removed after 24 hours exposure to *G. indica*, killed in 70% ethyl alcohol, dissected in 1% saline solution and examined using a Leica Stereo Microscope MZ6® at magnification x40. Based on Daane *et al.* (2004) some mobile mealybugs not showing outward signs of parasitism were identified for dissection by the small black scar tissue, a residual mark from the oviposition action. Dissection was performed by making a horizontal slit near the oviposition scar to expose the internal cavity. To ensure that all the eggs present were observed, the dissected mealybugs were examined using a Leica® Binocular Microscope with Phase Contrast (Model Galen III) at magnification x100. A similar batch was removed 12 hours later followed by a third batch another 12 hours later. Subsequent batches of ten were removed every 24 hours and dissected in the same manner to recover all the developmental stages of the parasitoid following the method of Odebiyi and Bokonon-Ganta (1986). This procedure was continued until Day 24 post exposure as most of the hosts were mummified indicating pupation of the larval stage of the parasitoid. This study was conducted at 28 ± 2°C and 60 ± 10% RH under a photoperiod of 8L:16D.

Life fecundity table for *G. indica*

A fecundity life table was constructed for *G. indica* by following a cohort of 100 pairs of mated individuals fed on *M. hirsutus* on sprouted potatoes. Sexes were differentiated on the basis of their antennae – male antennae had a narrow scape and a flagellum with long setae while the female had a much expanded scape and setae were significantly shorter. A sprouted potato infested with approximately 200 third instar *M. hirsutus* was placed in a transparent plastic container (12 cm × 7 cm × 9 cm) with a mesh covered top to allow for aeration and feeding with honey. A mated pair of *G. indica* was released in the container and left for 24 hours. The pair was removed and transferred to a similarly infested potato for 24 hours. This was repeated for all 100 pairs until

TABLE 1. Developmental time of immature stage of *Gyranusoidea indica* at 28 ± 2°C and 60 ± 10% RH under a photoperiod of 8L:16D.

Stage	*n*	Developmental time (days) Mean ± S.E.
Egg	30	2.08 ± 0.07
Larva	82	13.17 ± 0.14
Pupa	78	8.95 ± 0.33

the females of each pair died. In cases where the male died before the female, it was replaced by a male of similar age. Mummies formed on the sprouted potatoes from Day 14 were collected with a fine camel hair brush (size 0), placed in a 5 cm Petri dish and observed daily for parasitoid emergence. The number of mummies produced per day during the life of the female wasp; number and sex of emerging parasitoids and longevity of both sexes of parent parasitoids were recorded. This study was conducted at 28 ± 2°C and 60 ± 10% RH under a photoperiod of 8L:16D.

Based on Birch (1948), the net reproductive rate $R_o = \Sigma l_x m_x$ is the number of times a population will multiply per generation where l_x is the proportion of females alive at age x and m_x is the number of females born per female at age x Southwood and Henderson (2000); cohort generation time $T_c = \Sigma x l_x m_x / R_o$; finite rate of increase $\lambda = e^{rm}$ and the doubling time $DT = \ln 2 / r_m$ were calculated. The innate capacity for increase rm was calculated by trial and error substitution in the equation: $\Sigma e^{7-rm} x l_x m_x$.

RESULTS

Developmental biology of *G. indica*

The newly deposited parasitoid egg was translucent white; oval shaped and found lying freely in the host's body cavity. Normally one egg per host was found but two or more were sometimes encountered, with four being the maximum recorded. The egg incubation period lasted two to three days (Table 1). Post eclosion, the larva floated freely in the host's body cavity. The larval stage lasted 13.17 ± 0.14 days. The pupal stage continued within the host and changed the shape and texture of the host to a thin walled, barrel-shaped case, the mummy, which enclosed the developing parasitoid. The mummy was initially golden yellow but gradually changed to dark yellow and finally light brown. The pupal stage lasted 8.95 ± 0.33 days (Table 1). The adult eclosed by pushing its' self through the posterior end of the mummy case.

Life fecundity table for *G. indica*

The developmental success of the parasitoid from 3rd instar to adult was 33.3%. Mating occurred within the 24 hours that the male was placed with the female. The oviposition period was 6.71 ± 0.49 days and resulted in 50.13 ± 4.57 eggs. Thirty

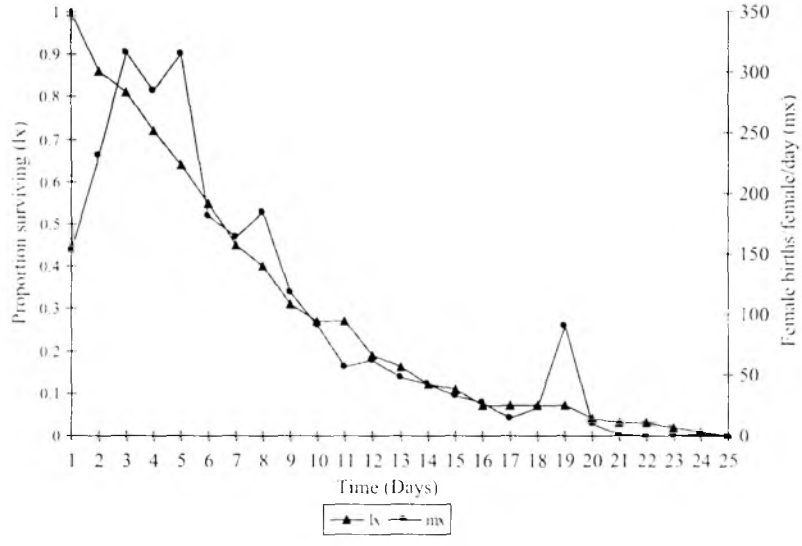
TABLE 2. Life fecundity table for *Gyranusoidea indica* at 28 ± 2°C and 60 ± 10% RH under a photoperiod of 8L:16D.

Stage	Age in days (x)	Proportion surviving (l_x)	Age schedule of female births (m_x)	$l_x m_x$	$x l_x m_x$
Immature	0–19	1.00	—	—	—
Adults	20	1.00	153	153.00	3060.00
	21	0.86	231	198.66	4171.86
	22	0.81	316	255.96	5631.12
	23	0.72	285	205.20	4719.60
	24	0.64	315	201.60	4838.40
	25	0.55	182	100.10	2502.50
	26	0.45	164	73.80	1918.80
	27	0.40	185	74.00	1998.00
	28	0.31	119	36.89	1032.92
	29	0.27	91	24.57	712.53
	30	0.27	56	15.12	453.60
	31	0.19	62	11.78	365.18
	32	0.16	48	7.68	245.76
	33	0.12	42	5.04	166.32
	34	0.11	33	3.63	123.42
	35	0.07	27	1.89	66.15
	36	0.07	14	0.98	35.28
	37	0.07	23	1.61	59.57
	38	0.07	90	6.30	239.40
	39	0.04	10	0.40	15.60
	40	0.03	1	0.03	1.20
	41	0.03	0	0.00	0.00
	42	0.02	0	0.00	0.00
	43	0.01	1	0.01	0.43
	44	0.00	0	0.00	0.00
				Σ 1378.25	Σ 32357.64

percent of the mated pairs did not produce any offspring on the 1st day that they were placed on the host. The adult sex ratio obtained was 1:1. The fecundity curve reached its peak on the 3rd day post adult eclosion (Fig. 1) thereafter the average number of female births/female/day (m_x) peaked several times on the 3rd, 5th, 8th and 19th day post eclosion (Fig. 1). Eighty two percent of adults eclosed within the first 10 days of the first parasitoid emergence. The age specific survival (l_x) of the parasitoid was high in the first six days post adult eclosion but declined sharply after 11 days. The first female death occurred on Day 2 and mortality increased afterward until death of the last female on Day 25 (Table 2). Female parasitoids lived for 7.11 ± 0.51 days while males lived for 4.18 ± 0.53 days (Table 3).

TABLE 3. Comparison of life table parameters of selected encrytid parasitoids

Parameters	*Gyranusoidea indica* (This study)	*Gyranusoidea tebygi* on *Rastrococcus invadens* (Boavida *et al.* 1995)	*Anagyrus kamali* on *Maconellicoccus hirsutus* (Persad and Khan 2002)	*Epidinocarsis lopezi* on *Phenacoccus manihoti* (Iziquel and LeRu 1992)
Temperature (°C)	28 ± 2	25	27 ± 3	26 ± 0.5
R.H. (%)	70 ± 10	83 ± 2	58 ± 3	64 ± 2
Net reproductive rate (♀/♀generation)	1378.25	51.8 ± 5.0	994.00	269.9
Cohort generation time (days)	23.48	–	20.50	–
Generation time (days)	21.98	27.3	20.05	33.9
Capacity for increase (♀/♀/day)	0.3079	–	0.3133	–
Innate capacity for increase	0.3289	0.145 ± 0.003	0.3298	0.213
Finite rate of increase (♀/♀/day)	1.3894	1.16	1.39	–
Doubling time (days)	2.11	4.8	2.09	–
Female longevity (days)	7.11 ± 0.51	–	7.23 ± 0.44	41.4 ± 15.5
Male longevity (days)	4.18 ± 0.53	–	5.94 ± 0.33	–



FIGURES 1. Fecundity (m_x) and survival (l_x) curves for Granusoidea indica at 28 ± 2°C and 7 ± 10% RH.

The net reproductive rate R_0 (number of females/female) for *G. indica* was 1378.25 (Table 2) while the number of females added to the female population per unit time (finite rate of increase λ), was 1.3894. The intrinsic rate of population increase was 0.3289. The mean generation time, T was 21.98 days while the population doubled itself in 2.11 days (Table 3).

DISCUSSION

Life tables are an important component in estimating the vital statistics of an insect population (La Rossa *et al.*, 2002). Based these studies the survival rate at each stage of the insect and the fecundity data of the females may be used to describe the population size of the insect and its age structure at any given time (Southwood, 1991). All females did not oviposit on the first day after mating. Additionally, parasitoids did not emerge from all cages on a daily basis. This irregular pattern of oviposition over its lifetime suggests that *G. indica* is synovigenic, a condition in which females periodically develop eggs during their adult life. Similar findings of synovigenic parasitoids have been reported in the literature for the aphelinids *Aphytis melinus* DeBach (Casas *et al.*, 2000), *A. aonidae* and *Encarsia pergandiella*, the braconid *Bracon hebetor* (Heimpel and Rosenheim, 1998) and the eulophid *Thripobius semiluteus* Bouček (Bernado *et al.*, 2005). Proovigeny or female emergence with the entire potential lifetime complement of mature eggs is a rare egg

TABLE 4. Comparison of life table parameters of *Gyranusoidea indica* and *Maconellicoccus hirsutus* on *Hibiscus rosa-sinensis*

Parameters	*Gyranusoidea indica* on *Maconellicoccus hirsutus* on *Solanum tuberosum* (This study)	*Maconellicoccus hirsutus* on *Hibiscus rosa-sinensis* (Persad and Khan, 2002)
Temperature (°C)	28 ± 2	27 ± 3
R.H. (%)	70 ± 10	58 ± 3
Net reproductive rate (♀/♀/generation)	1378.25	15.51
Cohort generation time (days)	23.48	–
Generation time (days)	21.98	34.23
Capacity for increase (♀/♀/day)	0.3079	–
Innate capacity for increase	0.3289	0.0801
Finite rate of increase (♀/♀/day)	1.3894	1.0800
Doubling time (days)	2.10	8.83
Female longevity (days)	7.11 ± 0.51	13.87 ± 2.25
Male longevity (days)	4.18 ± 0.53	2.73 ± 0.14

maturation strategy which is confined to a small number of species (Ellers and Jervis, 2004).

The net reproductive rate, R_0 or the number of times a population of *G. indica* multiplies per generation was 1378.25. In another congeneric species, *G. tebygi*, Boavida *et al.* (1995) reported a R_0 of 51.8 on the mango mealybug, *R. invadens*, while Iziquel and LeRu (1992) recorded 269.9 for *Epidinocarsis lopezi* on the cassava mealybug *Phenaccocus manihoti*. Persad and Khan (2002) determined the R_0 for *Anagyrus kamali* to be 994.0 on 3rd instar larval *M. hirsutus* under similar environmental conditions as the present study (Table 3). The generation time of *G. indica* of 22 days was also found to be within the range of these parasitoids it being closest to that of *A. kamali*. Comparison of data for *G. indica* from this study with that of other parasitoids from the literature suggests that *G. indica* has similar attributes as those of *A. kamali* under laboratory conditions. Their effect on the same mealybug host, *M. hirsutus*, is expected to be similar.

Life tables may be analyzed to determine the ecological role that natural enemies play in the host-natural enemy system (Bellows *et al.*, 1992). From life table parameters obtained for *M. hirsutus* by Persad and Khan (2002) under similar conditions it was concluded that R_0 of *G. indica* exceeded that of *M. hirsutus* by

a factor of about 88; also its generation time T was 0.6 times that of its host. The innate capacity for increase r_m of a parasitoid can be used in the absence of other criteria to select a candidate parasitoid species (Haque and Copland, 2002) and is the most important parameter obtained from a life table since it allows the comparison of the potential increase among species (La Rossa *et al.*, 2002). The innate capacity for increase, r_m of the parasitoid at 0.3289 was 4 times that of *M. hirsutus*. The higher r_m and lower generation time indicate that *G. indica* has a higher potential for population increase as compared to its host *M. hirsutus* (Table 4). These life table parameters suggest that *G. indica* is a good biological control agent of the hibiscus mealybug *M. hirsutus*. However, Neuenschwander (1996) cautioned that while detailed laboratory observations using life table studies can give very useful insights about the insect, it can describe its performance under sometimes ruefully inadequate conditions which may not allow the insect to demonstrate its typical potential. The effectiveness of beneficial insects predicted from laboratory results must therefore be supplemented by studies under natural conditions (Bigler, 1989).

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Effect of plant leaf extracts on the morphogenesis of *Tribolium castaneum* H.

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ABSTRACT: Treatment of *Tribolium castaneum* H. with the common medicinal plants *Lantana camara*, *Hyptis sauveolens*, *Citrus medica*, *Aegle marmelos* and *Lygodium flexosum* resulted in the formation of intermediate forms. The adult emergence was partially inhibited by the extracts. The formation of larval-pupal and pupal-adult intermediate forms was clearly dose dependant. The microscopic analysis shows the toxic properties of the extracts which causes morphogenetic changes in the insect. So it can be suggested that the plant extracts can be used as biopesticides against stored product insect *T. castaneum*. © 2011 Association for Advancement of Entomology

KEYWORDS: *Tribolium castaneum*, *Lantana camara*, *Hyptis sauveolens*, *Citrus medica*, *Aegle marmelos*, *Lygodium flexosum*, larval-pupal intermediate, pupal adult intermediate, bio pesticides

INTRODUCTION

Many studies are going on around the world, to screen the extracts of various plants for their insecticidal effect. If treated larvae do complete development they may give rise to morphologically deformed adults (Williams and Amos, 1974). They recognized a range of adult deformities with *Tribolium castaneum*. Some insect growth regulators increased mortality while others inhibited complete development of adults depending on the dose applied. Application of insect growth regulators to larvae leads to the production of these mosaics, and has been demonstrated in stored products insects by Strong and Diekman (1973); Loschiavo (1975); Metwally and Sehnal (1973).

When larvae are subjected to some of the growth regulators, it results in the disruption of pupal development and adult emergence and the formation of various intermediate forms between larva and pupa, pupa and adult or larva and adult, where reproduction is inhibited and normal ecdysis is not achieved. Cuticle synthesis and moulting are controlled by three groups of growth regulators—the brain hormones, the ecdysones and the juvenile hormones. Under normal concentrations, these growth

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regulators also control other developmental processes in insects including sexual maturation, colour differentiation and reproduction.

The important changes in development of insects are irrespective of growth. The differentiation may be chemical, morphological or functional. The growth and development inhibition in *Tribolium confusum* by the action of Solasodine is also reported by Barnby and Klocke (1990). Shalaby *et al.* (1998) reported abnormal adults in *Culex pipens*, when treated with oils of lemon, grape fruit and orange, which were not able to survive. Crook *et al.* (2008) indicated the presence of high juvenile hormone levels in the treated larvae. Not only have morphologically deformed adults been produced when exposed to various concentrations of Juvenile hormone analogues, but also adults whose reproductive physiology has been impaired in some way.

Inhibition of adult emergence was also observed in *Callasobruchus chinesis* treated with different vegetable oils (Khaire *et al.*, 1992) and *Vittalaria paradoxa* seed oil (Abdullahi *et al.*, 2011).

The present study deals with the morphogenetic changes produced in *T. castaneum* during the treatment with different plant leaf extracts of *L.camara, H. sauveolens, C. medica, A. marmelos* and *L. flexosum*.

MATERIALS AND METHODS

Test insect

Tribolium castaneum H.

Red flour beetles have chewing mouthparts, but do not bite or sting. The beetles are 1/4 inch long: the adults are long-lived and may live for more than three years. The eggs are white, microscopic and often have bits of flour stuck to their surface. The slender larvae are creamy yellow to light brown in colour. The pupae are lighter in colour, being white to yellowish. These beetles can breed throughout the year. The beetles give off an unpleasant smell, and their presence encourages mould growth in flour.

Culturing of test insects

Initial stock was obtained from infested flour bought from the local market. The adult insects were introduced into the raw rice and wheat flour. The culture was maintained in the laboratory. The cultures were cleaned everyday to avoid contamination. First and last instar larvae were collected for the experiments.

Treatments

Filter paper deposit method was used for the studies. No. 1 what man filter paper cut in to round shape was deposited in the bottles (containers with lid-diameter 5 cm, height-2.5 cm). The leaf extract diluted to different concentrations (1%, 2%, 3%, 4%, 5% and 10% respectively) were applied to the filter paper and allowed to dry. 20 gm fresh feed

(wheat flour) was taken in the container. 10 larvae were deposited in the container. Control was prepared in the same way. The extract application to the filter paper was omitted in the control. Instead of extracts, solvents were added. Five replicates were set up for the control and the treated. The development of the larvae were assessed and recorded every day, up to adulthood.

Instruments used or microscopic analysis

Digital camera, Dissection microscope, ESEM and Atomic force microscope have been used for the microscopic analysis of the intermediate forms formed after the treatment with the plant extracts.

OBSERVATION AND RESULTS

It was observed that adult emergence was inhibited in the *Tribolium castaneum*. The results showed that adult emergence is affected by the phytochemicals. Some of the pupae have not emerged into adults while few of them developed into, larval pupal intermediates and pupal adult intermediates, in which the characters of larvae, pupae and adult were present. Adult emergence was totally inhibited in these forms. Some of the pupae emerged into abnormal adults. These adults exhibited malformed wings, i.e. shorter wings when compared to normal. Some of them showed pupal case attached to the posterior region of the body (Figs.–).

Any abnormal shape, size, color or failure to pupate was indicated as malformation of larvae. All malformed larvae died after a short time, they were counted and removed immediately. Larval malformation percent was estimated by using the following equation: Larval malformation % = C/A × 100, where C = number of malformed larvae and A = number of tested larvae. The formation of intermediate forms was found to be dose dependant.

Pupal malformation was estimated by any change in color, size, shape or failure to develop to adult stage (pupal adult intermediate). All malformed pupae were counted and removed immediately. The pupal malformation percent was calculated by using the following equation: Pupal malformation % = C/A × 100, where C = number of malformed pupae and A = number of tested pupae.

The results have been given in Table 1. No malformation in development was observed in the lower doses tried. The percentage of malformation increased with the increase in dose.

DISCUSSION

The results show that adult emergence is affected by the phytochemicals, which often cause toxicity in the insects. These compounds exhibit effects on developmental stages of treated larvae, which can produce morphological abnormalities in different developmental stages such as lack of melanisation in larval and pupal stages, dead larvae-pupal intermediate stage with the head of pupa and the abdomen of a larva, dead adults with small wings in pupal exuvium and emerged adults unable to escape

TABLE 1. Showing the formation intermediate forms (%) in *T. castaneum*

Plants	Dose in %					
	1	2	3	4	5	10
L. camara	10	20	20	30	30	40
H. sauveolens	0	10	10	20	20	30
C. medica	0	0	10	20	30	30
A. marmelos	0	0	10	10	20	20
L. flexosum	0	10	10	20	30	40

the pupal exoskeleton, half ecdysed adults, formation of a mass of skin on the body of the insects, etc. Some of them showed pupal case attached to the posterior region of the body. This may prevent the insects from defaecation and mating. The malformed insects have shown aberrations of the legs reduced to un chitinised stump-like appendages, lack of differentiation and poorly chitinised antennae clubs, small diverging wings and elytra and developmental intermediates or "adultoids" (pupal-adult intermediates).

The present study clearly supports earlier reports stating that plant extracts contain compounds which act against insects in several ways. The formation of larval adult intermediates and pupal adult intermediates indicate that the hormonal levels of the treated insects may be affected. The inhibition of metamorphosis as a result of azadiractin application affected the emergence of pupae due to hormonal disturbance control and/or interference in chitin synthesis during the moulting process (Aldhous, 1992; Mordue and Blackwell, 1993). Similar results were obtained by Naqvi (1986), who found that treatment of *Aedes aegypti* larvae with natural fractions of neem leaves produced larval-pupal intermediates.

El-Shazly *et al.* (1996) reported similar findings on *Muscina stabulans* larvae treated with ethanol extract of *Nerium oleander* leaves. Ivbijaro (1986) tested neem ethanolic extract against same pest used in this study i.e. *T. castaneum*, and also found that the few pupae that survived metamorphosed into malformed adults. Abahussain (1999) studied the morphological effects induced by *Calotropis procera* in *C. pipiens* and *A. multicolor*, and again this produced larval-pupal intermediates and pupal adult intermediates. High frequencies of incomplete eclosion in adults within the individuals were also observed in this study. Complete eclosions with crumped wings or legs in adults were also found. Moursy (1997) and Hussein (2002) reported similar results on *Sarcophaga haemorroidalis* and *Culex pipiens* larvae respectively. The juvenile hormone and its analogues were proposed to play a significant role in regulating the reproduction (Prabhu and John, 1976; Jagajothi and Martin, 2010).

Sláma and Williams (1966) discovered that plant extracts, containing juvenile hormone analogue cause abnormal moulting and metamorphosis. In stored products insects, sterilizing effect has been observed in the "khapra beetle" Trogoderma granarium (Metwally and Lande, 1972; Chellayyan and Karanavar, 1990) as well as





FIGURES 1 & 2. Digital Photos:Larval—Pupal Intermediates.



FIGURE 3. SEM image of Control insect.

in *Tribolium castaneum* when adults were previously reared in our incorproating either the IGR methoprene or hydroprene (Amos and Semple, 1977). Leftwich (1976) has suggested delay in the appearance of adult characters due to excessive juvenile hormone by disrupting the hormonal balance between corpora allata and pro thoracic gland whereby the production of insect intermediates may be formed.

In several other studies also, the application of the extracts resulted the emergence of deformed adults from the treated pupa. The emergence of deformed adults and malformed intermediates in the present study suggest that the extracts interfere with mechanisms under-lying development which is hormonally regulated. This type of appearance of malformations were reported from azadirachtin treated *S. litura* (Rao and Subrahmanyam, 1987), plumbagin treated *Helicoverpa armigera* (Krishnayya and Rao, 1995).

Larva-pupal intermediates had a pupal cuticle which is usually tanned on the abdomen, and on the dorsal region of the head and thorax. The remaining parts became





FIGURES 4 & 5. SEM image of Insect after Treatment with leaf extracts.

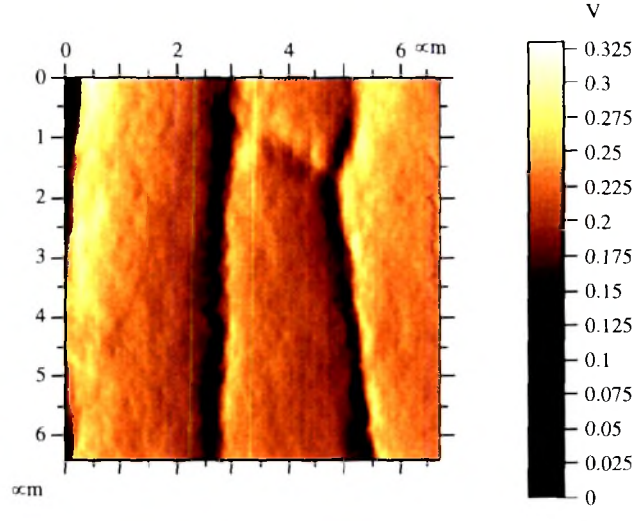


FIGURE 6. AFM image of Control insect.

darker when the insect was close to death. However no moult occurred and the body was still covered by the larval exuvium. None of the larvae were able to emerge. Similar results were reported when last instar larvae of *S. litura*, *S. mauritia*, *Ephestia kuehniella* Zell and *Manduca sexta* were subjected to azadirachtin (Jagannadh and Nair, 1992).

Morphogenic aberrations and abnormalities in larval, pupal, adult stages and intermediates of larval-pupal forms were increased signicantly with the increase in concentration as compared with control; these ndings are in agreement with those results obtained by Williams and Amos (1974) on *T. castaneum*. However, further investigation should be conducted in future to analyse the *mode* of action of the

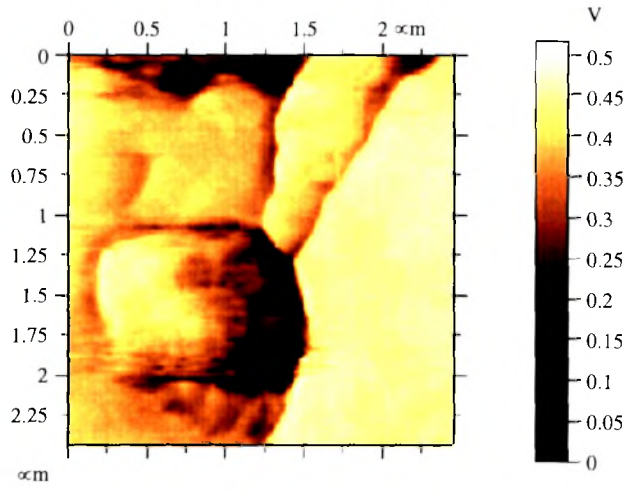


FIGURE 7. AFM image of Insect after Treatment with leaf extracts.

possible active components contained in *L. camara, H. sauveolens, C. medica, A. marmelos* and *L. fexosum* .

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Effect of some selected physical, chemical and biological stressors on the protein metabolism of *Oryctes rhinoceros* [L] Grubs

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ABSTRACT: *Oryctes rhinoceros* grubs on exposure to various stressors, such as extreme temperatures (5°C and 47°C) and intrahemocoelic injection of larvae with *Bacillus thuringiensis israelensis*, *Escherichia coli*, ovalbumin, casein and avian RBCs resulted hyperproteinaemia of the hemolymph. Reaction of *O. rhinoceros* grubs against experimental infection with entomopathogenic mite, *Poecilochirus sp.*, has also resulted in hemolymph hyperproteinaemia, evidenced by changes on the protein profile in SDS-PAGE. In all the above circumstances elevation of protein was co-related with the depletion in Total Free Amino Acids (TFAA) and elevation of uric acid. Healthy larvae always maintained a very high content of trehalose in the hemolymph which was almost ten times higher than that of the glucose content. Slight, but significant increase in glucose levels together with sharp elevation in the activity of Trehalase enzyme have resulted in hypotrehalosemia in the hemolymph under all the stress conditions. Both Aspartate Amino Transferase (AsAT/GOT) and Alanine Amino Transferase (AlAT/GPT) showed an elevation under all the above mentioned stress conditions. Behaviour of two proteolytic enzymes such as Cathepsin D and Leucine Amino Peptidase (LAP) were different under all the above mentioned stress conditions, which was evidenced by sharp elevation in Cathepsin D activity, while at the same time Leucine Amino Peptidase activity showed a sharp decline. How can these wonderful reactions by the hemolymph become helpful to the grubs during their life, in their ecological habitat filled with various types of organic molecules at different states of fermentation, needs a detailed study.
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KEYWORDS: *Oryctes rhinoceros*, Temperature stress, Hyperproteinaemia, Leucine Amino Peptidase, Cathepsin D, Trehalase.

INTRODUCTION

Insects are the most successful organisms in the planet earth, as they could easily cope up with the changes in the environment. Larval forms of highly injurious

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insect, mosquitoes and economically harmful insect pest *Oryctes rhinoceros* are successfully living and completing their life cycle in ecological conditions filled with millions of fermenting bacteria and full of noxious chemicals. Mosquito larvae can tolerate wide fluctuations of pH ranging from 4 to 10 (Gullan and Cranston, 1996). Grubs of *Oryctes rhinoceros* could be observed to be thriving well in compost pits with elevated temperature of about 38–42°C. The grubs fed with the cow dung contaminated with *Bacillus thuringiensis israelensis* (*Bti*) spores and intrahemocoelic injection of *Bti* endotoxin resulted in sharp increase in hemolymph protein levels as well as a drastic change in the differential and total hemocyte count (Adhira *et al.*, 2010). Exposure of *Oryctes rhinoceros* grubs to a variety of physicochemical and biological stressors, such as, temperature extremes, infestation by parasitic mites, intrahemocoelic injection of Ovalbumin, Casein, chick RBCs, *Bacillus thuringiensis israelensis* and *Escherichia coli* have resulted in sharp changes in the activity of various proteolytic enzymes, Transaminases and Trehalase present in the hemolymph forms the subject matter of the research communication.

MATERIALS AND METHODS

Healthy and actively feeding 3rd instar larvae of *Oryctes rhinoceros* collected from various compost and cow dung pits from in and around Trivandrum, Kerala were reared in plastic containers (11 cm × 8 cm) containing sterile cow dung, under laboratory conditions at an ambient temperature of 27±2°C (Sreekumar and Prabhu, 1988). Larvae having an average weight of 11.6±1.02 g were selected for the study.

Exposure of Insect Larvae to Various Stressors

Intrahemocoelic Challenge with Bacteria, Alien proteins and Chick RBCs. The larvae of *O.rhinoceros* were challenged with intrahemocoelic injection of live bacteria such as an entomopathogenic *Bacillus thuringiensis israelensis* [*Bti*], a non-entomopathogenic *Escherichia coli* [*E. coli*], alien proteins like Ovalbumin and Casein and chick RBCs. The site of injection was cleaned with 70% ethanol prior to all intrahemocoelic administration.

The bacterial strains of *Bti* and *E. coli* were cultured under sterile conditions according to standard microbiological protocols (Pelczar *et al.*, 1993). Live bacteria were administered into the larval body by intrahemocoelic injection, using a sterile insulin syringe, at a dose containing 10,000 bacteria/larvae.

Ovalbumin and casein dissolved in phosphate buffer solution (PBS) (pH: 7.2) were administered into the body of the larvae by intrahemocoelic injection using a sterile insulin syringe at a dose of 30 micrograms/larvae.

Chick RBCs washed and suspended in PBS were injected into the larval hemocoel at a dose of 4,000 cells/larvae.

In all the above three intrahemocoelic challenges, the hemolymph from the larval body was withdrawn for the study after twelve hours. All intrahemocoelic challenges were done after proper dose response study with at least 6 doses and in all such

injections the volume was limited to 30 l. Twelve larvae were taken for each of the above intrahemocoelic injections. Another group of ten larvae were injected with equal amounts of PBS and was taken as the control.

Exposure to cold and heat treatments Larvae reared in sterile cow dung at 27 2 C, were kept in experimental cages that were maintained at 5 C and 47 C for a period of three hours. Hemolymph was withdrawn after three hours. Twelve larvae were taken for each temperature treatments. A group of ten larvae were taken as control.

Mite Infection A parasitic mite, *Poecilochirus sp.*, found on the body surface of *O. rhinoceros* larvae were carefully separated from the larval body by using a smooth painting brush with minimum disturbance. These mites were transferred into fresh sterile cow dung at a density of 50 mites/100 g cow dung. The healthy larvae were released into the mite infested cow dung. Hemolymph was withdrawn for the study after ve days of infection.

Hemolymph from both the normal and infected larvae was collected, by cutting its 3rd prothoracic leg and gently squeezing its body and stored in sterile Eppendorf tubes in a deep freezer. Circulating hemocytes were separated from the hemolymph by centrifugation at 14,000 rpm at 4 C for 15 mins.

Enzyme Bioassays

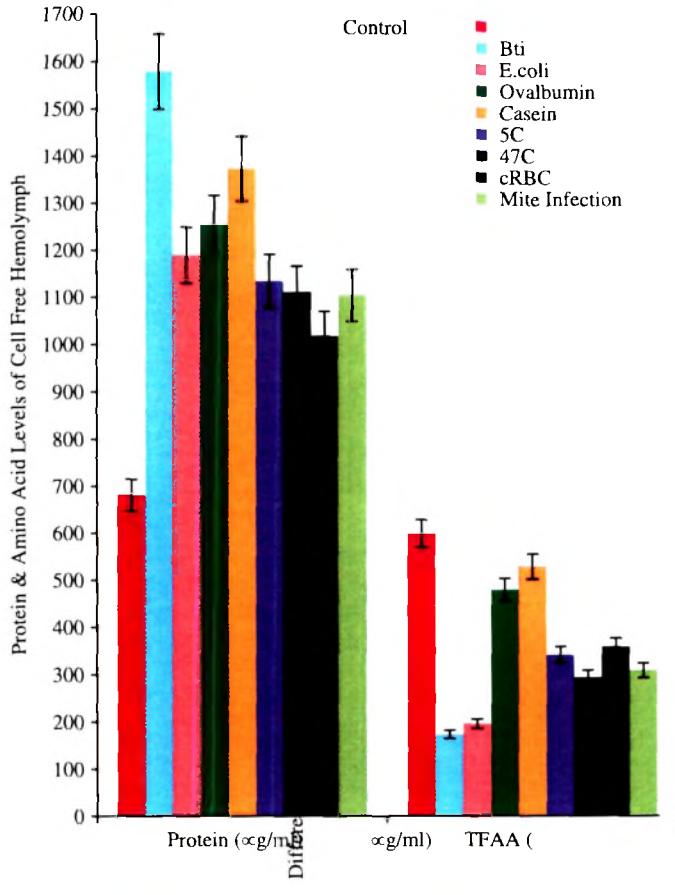
Bioassays of Protein (Lowry *et al.*, 1951), Total free Amino acids (Spies, 1957), Glucose (GOD/POD) (Trinder, 1969), Trehalose (Roe, 1955), Trehalase [E.C. 3.2.1.28] (Friedman, 1966), Transaminases (AsAT [E.C. 2.6.1.1] and AlAT [E.C. 2.6.1.2]) (Reitman and Frankel, 1957), Leucine Amino peptidase [E.C. 3.4.11.1] (Amador *et al.*, 1963) and Cathepsin D [E.C. 3.4.23.5] (Mycek, 1970) was carried out using standard protocols. Uric acid content of the cell free hemolymph was estimated using a standard assay kit purchased from Span diagnostics.

Electrophoretic studies

Electrophoresis of cell free hemolymph of larvae subjected to various stressors was carried out by the method devised by Laemmli (1970). The electrophorogram obtained was subjected to GEL-DOC Analysis using LABIMAGE PLATFORM software.

Statistical Analysis of data

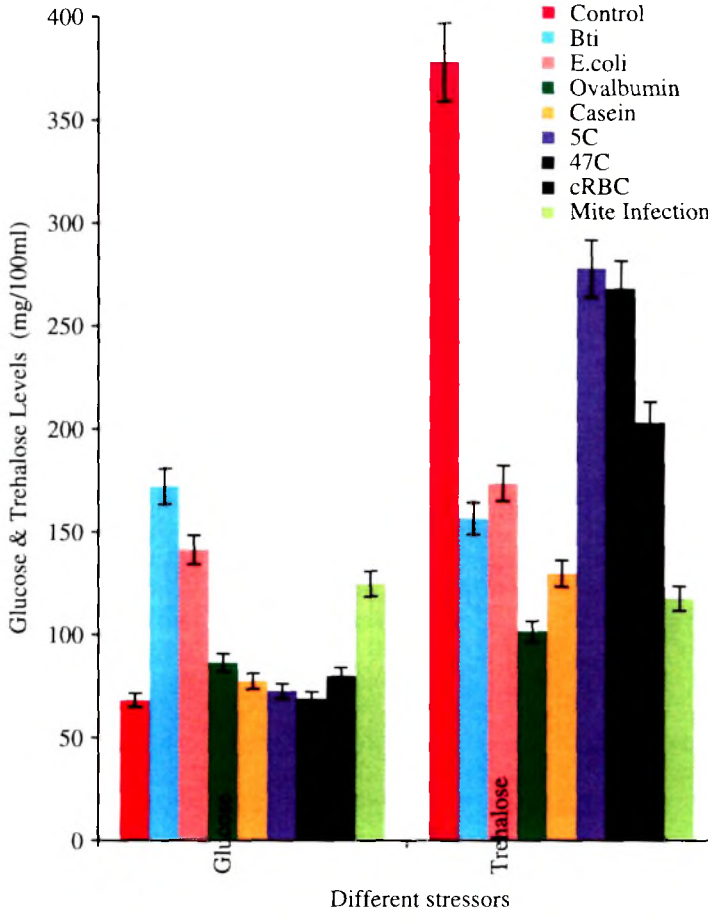
The data obtained are represented as Mean Standard Deviation. To test the signicance of the data obtained, statisti cal analysis were carried out using one-way ANOVA p 0 05 using SPSS software (Daniel, 2006).



FIGURES 1. Effect of various stressors on the content of Protein and TFAA in the hemolymph

OBSERVATION AND RESULTS

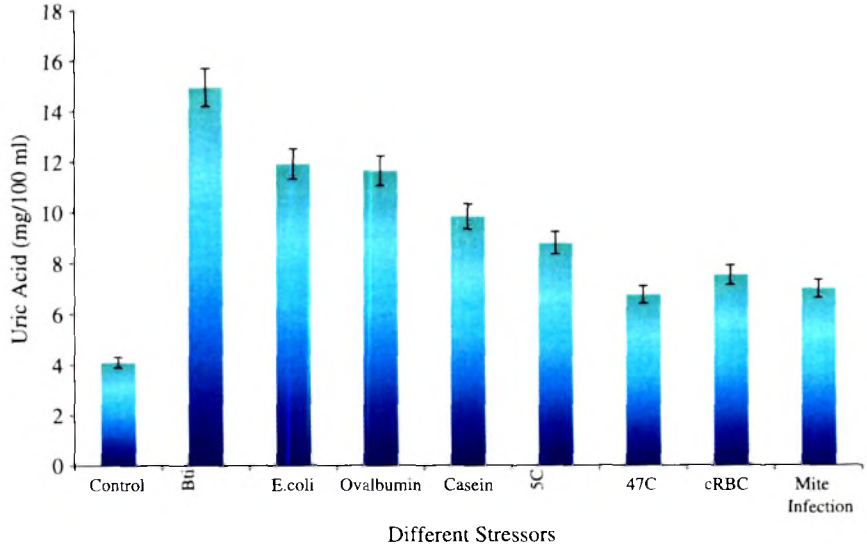
Intrahemocoelic injection of *O. rhinoceros* larvae with live bacteria, *Bti* and *E. coli* has resulted in a sharp elevation of the total protein together with the depletion of Total Free Amino Acids (TFAA). Two different proteins, Ovalbumin and Casein on intrahemocoelic injection resulted in a sharp elevation of hemolymph proteins higher than the 30 g quantity of alien protein that we have injected. Hyperproteinaemia and decreased TFAA have also been observed in the larvae, which have been administered with cRBCs. Five days of mite infection also resulted in an elevation of the hemolymph protein levels and decline in TFAA. Exposure of the larvae to 47 C and 5 C also showed a sharp increase in protein levels together with a steep decline in TFAA. Similar results were also obtained on intrahemocoelic administration of cRBC and it will be clear from Fig. 1.



FIGURES 2. Effect of various stressors on the content of Glucose and Trehalose in the hemolymph

Healthy larvae maintained very high content of trehalose when compared with glucose content which was seven times higher than that of hemolymph glucose content. Hyperglycemia and decline in trehalose levels were observed in the larvae exposed to 47 C and 5 C. Five days of mite infection also resulted in an elevation of glucose and hypotrehalosemia. Administration of two different proteins, Ovalbumin and Casein also caused signicant increas e in glucose levels together with decreased trehalose content. Similar results were observed on intrahemocoelic injection of live bacteria as well as with that of cRBCs and the results are shown in Fig. 2.

Elevation in uric acid levels upto four times than the normal was observed among the larvae in case of intrahemocoelic injection with live bacteria. Mite infected larvae



FIGURES 3. Effect of various stressors on the Uric Acid content of the hemolymph

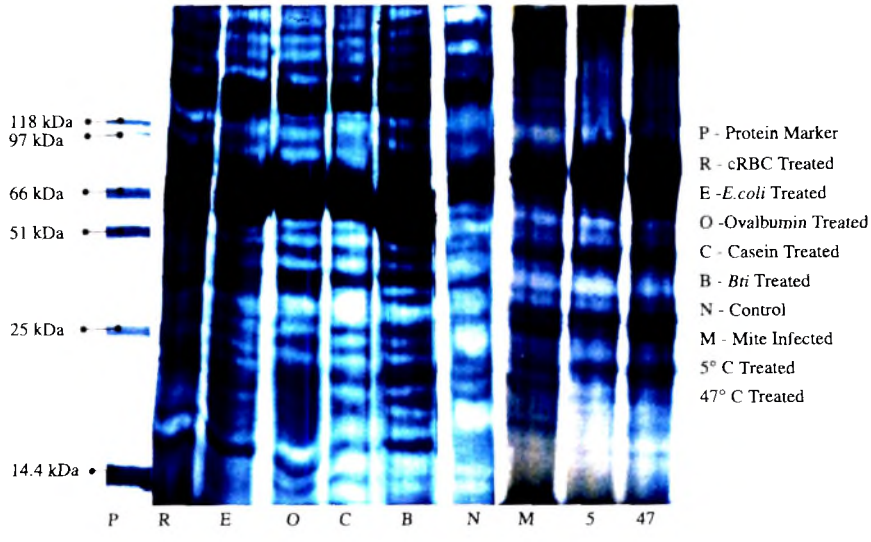


PLATE 1. Electrophorogram of *O. rhinoceros* Hemolymph in Response to Various Stressors

also showed a similar increase of uric acid in the hemolymph which yielded similar results on intrahemocoelic challenge (Fig. 3).

TABLE 1. Activity of various enzymes of *Oryctes rhinoceros* Larvae on Exposure to Various Stressors

Different Stressors	Transaminases		Cathepsin D (Units/μg Protein)	Leucine Amino Peptidase**	Trehalase (Units/mg Protein)
	AsAT/GOT (I.U/L)	AlAT/GPT (I.U/L)			
Control	151.19±13.34	42.14±3.61	0.483±0.035	167.35±14.23	19.065±1.32
Bti	235.2±21.08	120.72±11.47	0.802±0.068	31.66±2.87	30.286±2.67
E. coli	196.34±17.46	102.95±9.54	0.648±0.060	48.04±3.69	24.885±1.87
Ovalbumin	168.56±14.16	94.58±8.46	0.568±0.046	34.54±2.41	33.861±2.73
Caesin	163.85±13.91	98.43±8.94	0.564±0.044	31.88±2.36	28.441±2.46
5°C	191.71±16.83	105.11±9.67	0.577±0.051	58.72±4.75	23.305±1.75
47°C	179.62±14.67	95.85±8.61	0.587±0.054	69.71±5.87	22.812±1.58
cRBCs	172.08±14.27	99.31±8.73	0.572±0.039	63.42±5.84	22.630±1.51
Mite Infection	178.07±14.92	101.66±9.41	0.581±0.041	52.68±4.66	34.595±2.87

** Activity is expressed in Amount of β-naphthylamine liberated/hour/μg protein.

*All values are Mean ± Standard Deviation.

Activity of Transaminases, both AsAT/GOT and AlAT/GPT, showed a marked elevation on exposure to all the above cited stressors (Table 1). Activity of Trehalase, a major carbohydrate metabolizing enzyme, also showed a similar response in presence of the above mentioned stressors (Table 1).

Response of two proteolytic enzymes such as Leucine Amino Peptidase and Cathepsin D was always different under all the above mentioned stress condition (Table 1). In all the above mentioned stress conditions, the activity of Cathepsin D showed a sharp elevation while the activity of LAP showed a steep decrease.

Electrophorogram of the hemolymph under all the above described stress conditions showed the presence of additional protein bands and also significant thickening on existing bands (PLATE: I).

DISCUSSION

Oryctes rhinoceros larvae when subjected to various physical, biological and chemical stressors exhibited hyperproteinaemia, hyperglycemia and hyperuricemia together with a decrease in the levels of Total free amino acids. Elevated levels of protein indicate the de novo synthesis of novel proteins to overcome the stress. Similar observations were reported by other investigators among different insects in response to bacterial, temperature, antigenic stresses etc.. (Lakshmi and Rao, 2010; Denlinger and Lee, 2010; Nathan, 2006; Tripathi and Singh, 2002; Radha and Dwijendra, 1979). Hyperproteinaemia has also been observed in our lab studies in case of mosquito larvae and other insect larvae exposed to various environmental and biological stressors as well as in treatment with phytochemicals (Unpublished Observations). The total free amino acid level (TFAA) in the hemolymph of a healthy larva was very high and this condition was considered to be an important character of the Class Insecta (Evans and Kaleysa Raj, 1991, 1992). Contrary to this elevated levels of TFAA, exposure of the larvae to varying stressors caused a sharp decline of the same in the hemolymph. A sudden decrease in the TFAA levels has been reported in *Spodoptera litura* and *Phthorimea operculella* larvae (Salama *et al.*, 1994) and in *Plodia interpuctella* (Aboul-Ela *et al.*, 1991) when subjected to bacterial and temperature stress, as well as in parasitized insect larvae (Zhao, 1997). The low levels of TFAA indicate a disturbance in the metabolism of the larva. It was reported that maintenance of very high content of TFAA in the insect hemolymph has a major role in supplying metabolites for energy needs (Bailey, 1975). A sharp decline in TFAA together with the elevation of uric acid level up to 200–300% of its normal value was a clear indication of increased catabolism of TFAA in response to stressors. Decrease in the free amino acid content indicates the possibility of active catabolism of amino acids through transamination and subsequent entry of corresponding keto acids into the Kreb's cycle to meet the emergent energy requirement as well as their utilization in the production of some new proteins synthesized to cope up with the stress. Hyperproteinaemia of the hemolymph was also a pathological change observed in *O. rhinoceros* larvae on exposure to conditions of stress.

Hemolymph glucose of normal *O. rhinoceros* grubs was comparable with that of normal fasting blood glucose levels of healthy humans, but trehalose content was maintained at a very high concentration of 378 mg/100 ml. Maintenance of such a very high concentration of this disaccharide in the hemolymph of healthy grubs may have some other role in metabolism, besides simply acting as a glucose supplier. Hyperglycemia could be observed in the hemolymph of grubs under all the experimental stress conditions. Hyperglycemia may be due to the inhibition of glycolytic enzymes on exposure to different physicochemical and biological stresses. Similar results are reported by other investigators in silkworm, honey bees and cockroaches on exposure to varying stresses (Fast and Angus, 1965; Bendahou *et al.*, 1999). Hyperglycemia together with decreased trehalose contents indicated that glucose is not the immediate source of energy and insects rely mostly on other accessory energy resources, such as amino acids and fatty acids, during the stress conditions.

Hyperuricemia and hyperproteinaemia together with decrease in TFAA have pointed out the possibility of potential damage to the next nitrogenous component of the cell, particularly the nucleic acids, evidenced by the disintegration of nucleus of hemocytes (Adhira *et al.*, 2010). Increased catabolism of nucleotides may also contribute to hyperuricemia (Babu and Subramaniam, 1973; Tripathi and Singh, 2002).

The activity of hemolymph transaminases showed a significant elevation on all experimental stress conditions. Elevation in the activity of Transaminases in insects on infection with bacteria, on exposure to cold stress and parasitized conditions have been reported in other insects such as *Spodoptera litura* and corn borers (El-Shershaby, 2008; Salama *et al.*, 1999). Elevated activity of transaminases was also observed in mammalian systems during various pathological conditions (Subramanium *et al.*, 1998).

The proteolytic enzymes, Leucine aminopeptidase and Cathepsin D, differed in their activity in response to different stresses. Leucine Aminopeptidase, an exopeptidase, showed a decrease in its activity when the larvae were exposed to various stressors. On the other hand, Cathepsin D, an aspartic protease, showed an elevation in its activity under the influence of physico-chemical and biological stressors. Similar results were reported by various investigators in the insects exposed to different phytochemicals and synthetic insecticides such as Cyfluthrin (Saleem *et al.*, 2004, 2010).

Appearance of new protein bands and excess production of existing proteins in the hemolymph, as evidenced by SDS-PAGE electrophorogram, under all the experimental stress conditions indicates that insects responded effectively to all types of stressors such as physical, chemical and biological challenges through the up-regulation and/or down-regulation of genes.

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Preliminary survey of ant fauna (Hymenoptera: Formicidae) in Shenduruny Wildlife Sanctuary and nearby area, Kollam, Kerala

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ABSTRACT: Preliminary survey of ant fauna carried out for 2 days from Shenduruny Wildlife Sanctuary and nearby areas of Thenmala of Kollam district. The study location was mainly included 2 areas, the forest and the disturbed areas surround the forest by human activities. A total of 17 species from 11 genera and 4 subfamilies were observed. From the survey, Ponerinae and Formicinae were dominant subfamilies. From the survey most of the ant fauna dominated in the base region of the forest than top and most of the species were aggressive nature.
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KEYWORDS: ant fauna, Shenduruny Wildlife Sanctuary, Thenmala, Ponerinae, Formicinae, aggressive nature

INTRODUCTION

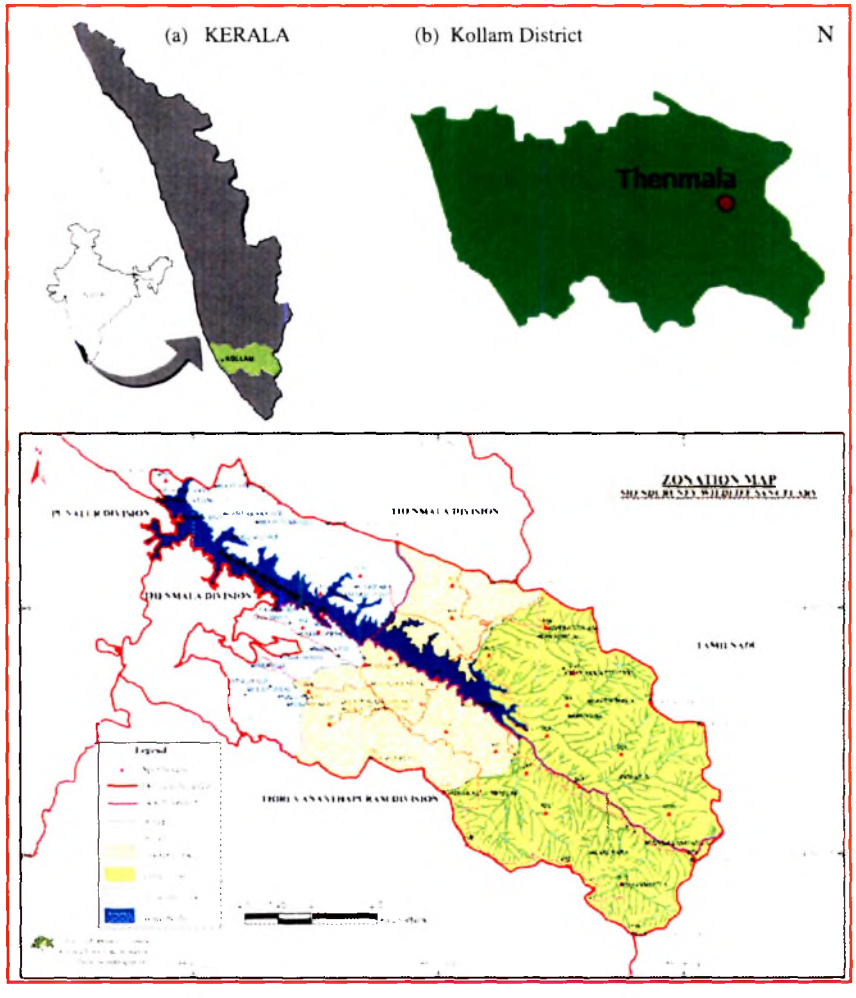
The etymological meaning of this Wildlife Sanctuary has driven from 'Shenkuruny' a tree species (*Gluta travancorica*), which is an endemic tree species conned to this area. Shenduruny Wildlife Sanctuary is situated in Thenmala (8 50 -8 55 North and 77 05 –77 15 East), Kollam district, Kerala. The Sanctuary situated Southern part of Western Ghats, with an altitude of 5633.2 ft. The forest mainly experience tropical evergreen and is suitable for insect fauna (Tiwari, 1999). Since the importance of ants has signicant role on environment in direct or indirect ways, the importance of the study on ants is very essential for biologists.

MATERIALS AND METHODS

A Survey of common ants were carried out in an area of Shenduruny Wildlife Sanctuary and nearby disturbed area of Thenmala (8.96N 77.13E). For the study, 14

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FIGURES 1. (a) Location of Kollam in Kerala, India; (b) Thenmala Region in Kollam; (c) Map of Shenduruny Wildlife Sanctuary.

areas were selected. Out of these 4 areas were disturbed and the remaining 10 were the undisturbed areas of Wildlife sanctuary. The specimens were collected by specially designed sweep net, all out search and brush methods. The collected specimens were mounted on a rectangular card measuring 14 mm in length and 5 mm width and was held on Entomological pins (Asta stainless steel insect pin, 38 mm .53 made by Newey Goodman Ltd. UK) for further studies. The mounted specimens were observed using Stereoscopic Binocular Microscope Model GS-44 Genter India and Lecia MZ6 stereo zoom microscope. Identication w ere carried out with the help of keys of Bingham (1903), Bolton's Key (1994) and Karmaly and Narendran (2006).

TABLE 1. List of Ant fauna observed from the Sanctuary and the nearby area of the sanctuary

Ant found in nearby areas of sanctuary	Ant found in Shenduruny Wildlife Sanctuary
1. *Oecophylla smaragdina* (Fabricius)	1. *Oecophylla smaragdina* (Fabricius)
2. *Anoplolepis gracilipes* (Smith)	2. *Anoplolepis gracilipes* (Smith)
3. *Solenopsis geminata* (Fabricius)	3. *Solenopsis geminata* (Fabricius)
4. *Myrmicaria brunnea* (Saunders)	4. *Myrmicaria brunnea* Saunders
5. *Camponotus parius* (Emery)	5. *Camponotus parius* Emery
6. *Camponotus mitis* (Smith)	6. *Camponotus binghamii* Forel
7. *Camponotus rufoglaucus* (Jerdon)	7. *Diacamma assamense* Forel
8. *Diacamma sculptum* (Jerdon)	8. *Diacamma ceylonense* Emery
9. *Meranoplus bicolor* (Guerin)	9. *Leptogenys dentilobis* Forel
10. *Pachycondyla luteipes* (Mayr)	10. *Leptogenys ocellifera* Roger
	11. *Odontomachus haematodes* (Linn.)
	12. *Technomyrmex albipes* (Smith)

Observations and results

A total of 4 subfamilies of Formicinae, Ponerinae, Myrmicinae and Dolichoderinae were found during study. The overall observation of ant population showed that the decline as goes inside and higher region and was densely populated in low and base region of the sanctuary. The other important feature was found that 70% of species were aggressive in nature.

In Subfamily Formicinae, a total of 7 species were observed. 3 species such as *Oecophylla smaragdina* (Fabricius), *Anoplolepis gracilipes* (Smith) and *Camponotus parius* Emery were common in both areas while *Camponotus binghamii* Forel observed only from the forest. *Camponotus mitis* (Smith) and *Camponotus rufoglaucus* (Jerdon) observed only from low region of the forest while *Camponotus mitis* (Smith) was found to inhabit close to human activities.

Most of the forest floor dominated by subfamily Ponerinae, groups and the nests were found on the open hard soil of the forest floor. From 7 species of Subfamily Ponerinae, 5 species were found from forest floor and were *Diacamma assamense* Forel, *Diacamma ceylonensis* Emery, *Leptogenys dentilobis* Forel, *Leptogenys ocellifera* Roger, *Odontomachus haematodes* Linnaeus. *Diacamma sculptum* Jerdon was found from the disturbed area. *Pachycondyla luteipes* (Mayr) was the other Ponerinae group, that was less populated and was found away from forest.

In subfamily Myrmicinae, *Solenopsis geminata* (Fabricius) was common in both areas, on soil and trees. *Myrmicaria brunnea* Saunders which were observed mainly on soil and the nest found on soil under tree. *Meranoplus bicolor* (Guérin), which was less dense groups and identified from the low region in nearby areas of forest.

Technomyrmex albipes (Smith) was the only species found in the study area belong to the subfamily Dolichoderinae. They were sparsely populated in study location.

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Comparison of external male genitalia of ten species of the genus *Homoeocerus* Burmeister from India (Hemiptera: Coreidae)

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ABSTRACT: Genus *Homoeocerus* Burmeister is widely distributed in the Oriental, Ethioian and Palaerctic regions. In this paper comparison of external male genitalia of ten species has been made on the basis of which species can easily be differentiated. A key to the studied species is also provided. Detailed descriptions of male genitalia of these species are given along with photographs.
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KEYWORDS: *Homoeocerus*, India, external male genitalia

INTRODUCTION

The genus is distributed in the Ethiopian, Oriental and Eastern Palaearctic regions. It is concentrated in the Oriental region and is fairly represented in the Ethiopian region. According to Fauna of British India (Distant, 1902, 1909, 1910, 1911, 1918) 49 species have been reported from British India. There is gradual transition from species having the pronotal angles well developed, with long and slender antennae, to others in which those angles are not prominent and combined with much shorter antennae, last joint of which tends to become shortened and thickened. The body also, from being elongate and straight, approaches a shorter and more laterally dilated formation. Species of this genus are very host specific and generally feed on *Acacia* plants. Here ten species of this genus have been compared for external genitalia. The male genitalia consists of pygophore, paramere and aedeagus which differ in different species on the basis of shape, size and number of various appendages.

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MATERIALS AND METHODS

Bugs were collected with the help of insect net from leaf litter and by beating vegetation and branches of trees below which a white sheet was spread. After collection, the bugs were killed with ethyl acetate vapours and pinned, stretched and kept in wooden boxes for further examination. For dissection of male and female genitalia the dried specimens were relaxed by keeping them in a pertidish under humid conditions for few hours. Their abdomens were separated from the whole insect and were kept in 10% KOH at 70°C for 40–80 minutes depending upon the size of the insect. After dissections the structures were washed in distilled water and then dehydrated through ascending grades of alcohol, cleared in clove oil and preserved. Photography was done using image processing unit located in the Department of Zoology and Environmental Sciences, Punjabi University, Patiala. Orientation of the genital structures was arranged in such a way that it clearly depicted its distinctive features. All the measurements have been made under stereozoom binocular microscope fitted with a graph eye piece (ocular grid).

Genus *Homoeocerus* Burmeister

Homoeocerus, Burmeister 1835, *Handb. ii*, **1**: p. 316.
Ceratopachys, Westwood 1842, *in hope Cat.*, **2**: p. 22.
Prismatocerus, Amyot and Servile 1843, *Hemiptera*, p. 185.
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Ornytus, part, Dallas 1852, *List Hem*, **2**: pp. 438 and 447.
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Type species: *Homoeocerus puncticornis* Burmeister.

Key to the studied species of genus *Homoeocerus* Burmeister on the basis of external male genitalia

1. Pygophore lobed 2
   Pygophore not lobed 6
2. Pygophore bilobed 3
   Pygphore trilobed 4
3. Ventroposterior margin or lobes of pygophore much broad ..... *borealis* Distant
   Ventroposterior margin or lobes of pygophore very narrow ..... *sigillatus* Stal
4. All the lobes of pygophore equal sized ..... *macula* Dallas
   Middle lobe of pygophore longer than lateral lobes 5
5. Lobes of the pygophore with partition, pygophore rectangular with ventroanterior margin substraight, paramere with short squarish stem and blade long, curved and slender, outer and inner margins sinuate, stem squarish with inner and outer margins sinuate ..... *atkinsoni* Distant

Lobes of the pygophore without partition, pygophore elongate with ventroanterior margin little sinuate, paramere with stem rectangular, blade short and thick with subround apex, outer and inner margins subround, blade and stem with a deep concavity, stem rectangular with inner margin straight .... fasciolatus Stal

6. Ventroposterior margin of pygophore much narrower than anterior margin 7
   Ventroposterior margin of pygophore subequal to anterior margin 8
7. Pygophore as long as broad, outer margin prominent sinuate, ventroposterior margin or lip narrow; paramere with stem broad, blade long and thick with narrow apex, margin of apex subround straight with a narrow process, outer and inner margins sinuate ..... *striicornis* Scott
   Pygophore much longer than broad, outer margin subround, ventroposterior margin or lip substraight; paramere with stem narrow, blade short, thick, slender and narrowed at apex with broad subround apical margin .. *serrifer* (Westwood)
8. Outer margin of pygophore sinuate ..... *lacertosus* Distant
   Outer margin of pygophore not sinuate 9
9. Outer margin of pygophore round, ventroanterior margin triangular, round up to posterior margin, ventroposterior margin or lip narrow, medially sinuate; paramere with short stem, blade thin and longer ..... *prominulus* (Dallas)
   Outer margin of pygophore substraight, ventroanterior margin substraight, substraight up to middle, then obliquely straigtht, ventroposterior margin or lip broad and subround; paramere with broad stem, blade thicker and shorter .... *signatus* Walker

Descriptions of the male genitalia of the studied species of *Homoeocerus* Burmeister:

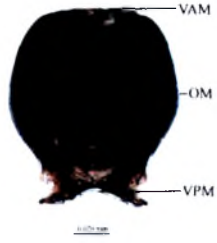
Homoeocerus borealis Distant:

Pygophore (Fig. 1) more or less rectangular, outer margin subprominent and subround, medially round and prominent and narrow and subround posteriorly, ventroposterior margin or lip broad and bilobed with triangular lobes having subround apices; paramere (Fig. 2) with outer margin subround and medially subprominent with long and thick blade, apex broad with round outer margin, a small acute process on inner margin, outer and inner margin little sinuate, stem squarish, inner margin with concavity; aedeagus (Figs. 3) with phallosoma large and wide especially in proximal region; conjunctiva with a pair of semisclerotized membranous appendages with broad truncate apex, vesica thin and not much coiled, ejaculatory reservoir round, basal plate with pivot.

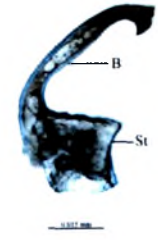
H. sigilattus Stal:

Pygophore (Fig. 4) squarish with outer anterior margin substraight up to middle and posteriorly becoming steeply narrowed, ventroposterior margin or lip narrow and bilobed with lobes round, hair near posterior margin; paramere (Fig. 5) with outer margin medially subprominent and round, blade long and slender, gradually

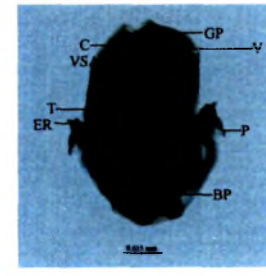
Homoeocerus borealis Distant



(1) Pygophore

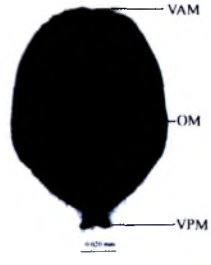


(2) Paramere

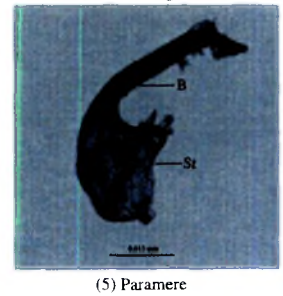


(3) Aedeagus

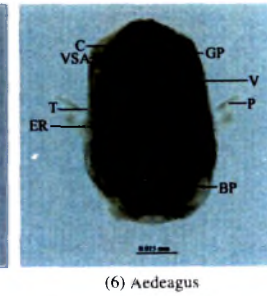
Homoeocerus sigillatus Stal



(4) Pygophore

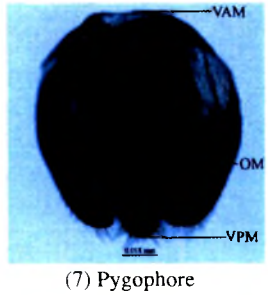


(5) Paramere

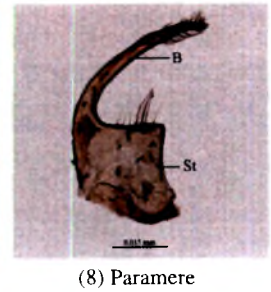


(6) Aedeagus

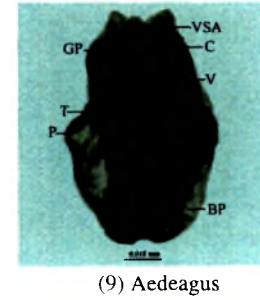
Homoeocerus macula Dallas



(7) Pygophore

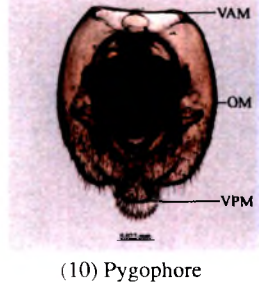


(8) Paramere

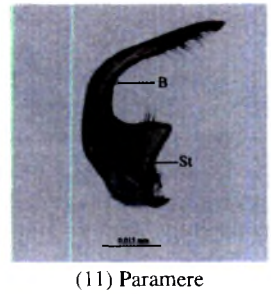


(9) Aedeagus

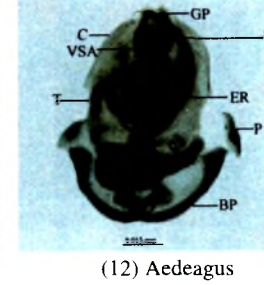
Homoeocerus atkinsoni Distant



(10) Pygophore



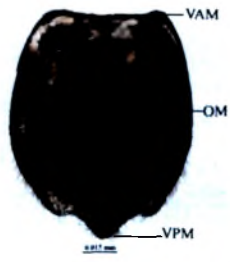
(11) Paramere



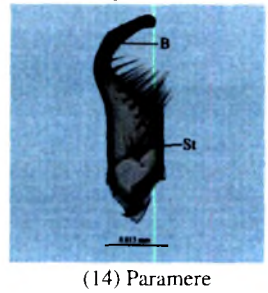
(12) Aedeagus

FIGURES 1–12. *Homoeocerus borealis* (Pygophore-1, Paramere-2, Aedeagus-3); *Homoeocerus sigillatus* (Pygophore-4, Paramere-5, Aedeagus-6); *Homoeocerus macula* (Pygophore-7, Paramere-8, Aedeagus-9); *Homoeocerus atkinsoni* (Pygophore-10, Paramere-11, Aedeagus-12).

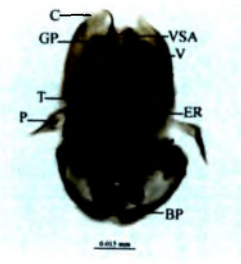
Homoeocerus fasciolatus



(13) Pygophore

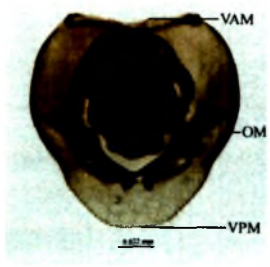


(14) Paramere

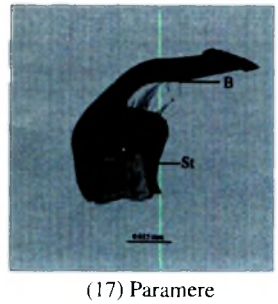


(15) Aedeagus

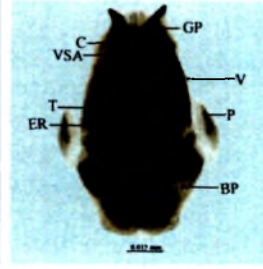
Homoeocerus striicornis Scott



(16) Pygophore

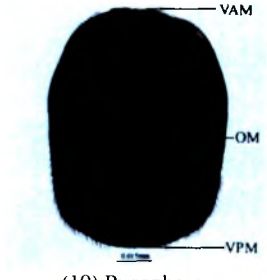


(17) Paramere

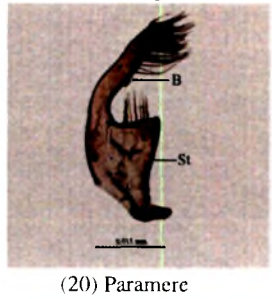


(18) Aedeagus Dallas

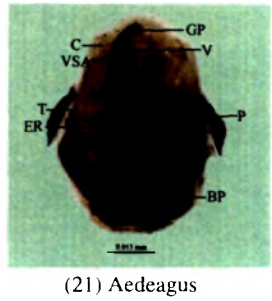
Homoeocerus serrifer (Westwood)



(19) Pygophore

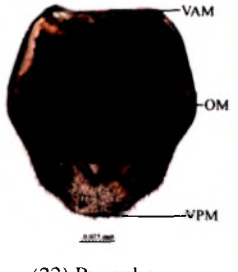


(20) Paramere

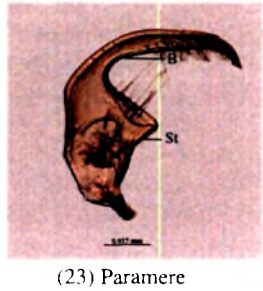


(21) Aedeagus

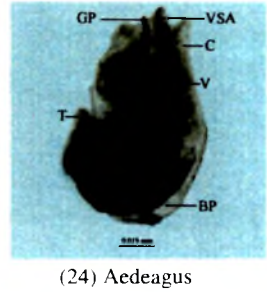
Homoeocerus lacertosus Distant



(22) Pygophore



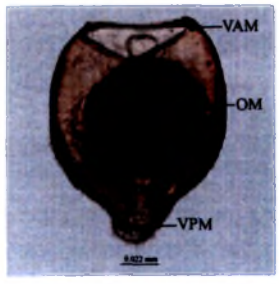
(23) Paramere



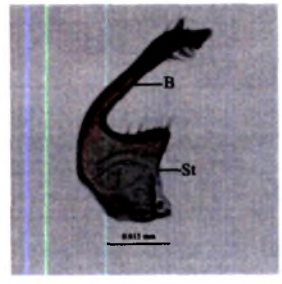
(24) Aedeagus

FIGURES 13–24. *Homoeocerus fasciolatus* (Pygophore-13, Paramere-14, Aedeagus-15); *Homoeocerus striicornis* (Pygophore-16, Paramere-17, Aedeagus-18); *Homoeocerus serrifer* (Pygophore-19, Paramere-20, Aedeagus-21); *Homoeocerus lacertosus* (Pygophore-22, Paramere-23, Aedeagus-24).

Homoeocerus prominulus



(25) Pygophore

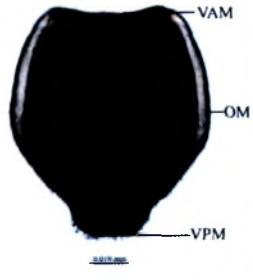


(26) Paramere

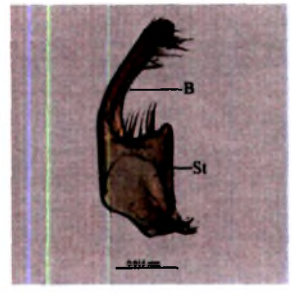


(27) Aedeagus

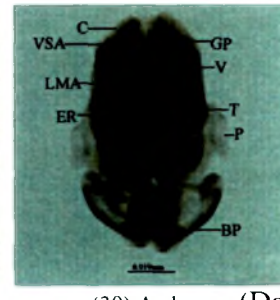
Homoeocerus signatus Walker



(28) Pygophore



(29) Paramere



(30) Aedeagus (Dallas)

FIGURES 25–30. *Homoeocerus prominulus* (Pygophore-25, Paramere-26, Aedeagus-27); *Homoeocerus signatus* (Pygophore-28, Paramere-29, Aedeagus-30).

narrow with broad substraight apical margin, outer and inner margins substraight, stem squarish with a small concavity on inner margin; aedeagus (Fig. 6) with phallosoma large and wide, conjunctiva with a pair of semisclerotized ventral appendages with subround apex, basal plate with pivot, vesica moderately thick with two loose coils.

H. macula Dallas:

Pygophore (Fig. 7) more or less oblong with ventroanterior margin straight, outer margin at anterior side round and broad, posteriorly narrow and substraight, ventroposterior margin with two lateral and one median lobe, medial lobe round and lateral lobes subround, posterior margin with hair; paramere (Fig. 8) with outer margin round medially, blade long, thick and narrow toward apex which is triangular in shape, outer and inner margins subround, slightly sinuate, stem squarish with inner margins forming concavity, hair on stem and blade; aedeagus (Fig. 9) with phallosoma large and wide, a pair of ventral appendages which are thin and highly sclerotized with narrow apex, vesica moderately thick with three coils, basal plate with pivot.

H. atkinsoni Distant:

Pygophore (Fig. 10) rectangular with ventroanterior margin substraight, outer lateral margin subround, ventroposterior margin or lip divided in three lobes, two narrow lateral and one broad median; paramere (Fig. 11) with outer margin medially subround, blade long, curved and slender with substraight apex, outer and inner margins sinuate, stem squarish with inner and outer margins sinuate; aedeagus (Fig. 12) with phallosoma large and wide, conjunctiva with a pair of ventral sclerotized appendages, vesica long and thin not much coiled, basal plate with pivot

H. fasciolatus Stal:

Pygophore (Fig. 13) elongate with ventroanterior margin little sinuate outer margin subround, ventroposterior margin or lip trilobed, two lateral lobes subround, middle narrow and triangular, hair on posterior region; paramere (Fig. 14) with medially subprominent and round outer margin, blade short and thick with subround apex, outer and inner margins subround, blade and stem with a deep concavity, stem rectangular with inner margin straight, hair very dense on stem and blade; aedeagus (Fig. 15) with phallosoma large and wide, conjunctiva with a pair of small sclerotized ventral appendages, a pair of wide ventrolateral appendages also present vesica thin with two coils, basal plate with pivot.

H. striicornis Scott:

Pygophore (Fig. 16) anteriorly very broad, outer margin prominent and round, sinuate after middle and then posteriorly round and narrow, ventroposterior margin or lip subround and narrow, hair on posterior region; paramere (Fig. 17) with sinuate outer margin, medially substriaght, blade long and thick with narrow apex, margin of apex straight with a narrow process, outer and inner margins sinuate, stem squarish with inner margin concave, hair on stem and blade; aedeagus (Fig. 18) with phallosoma large and elongate, ventral appendages thin, sclerotized with narrow apex on ventolateral side two broad appendages with subround narrow apex present, vesica thick with two coils, basal plate with pivot.

H. serrifer (Westwood):

Pygophore (Fig. 19) more or less rectangular, anterior outer margin subround and subprominent, posteriorly substraight, as long as broad, ventroposterior margin or lip substraight, hair on posterior side; paramere (Fig. 20) with outer margin subround and medially prominent, blade short, thick, slender and narrowed at apex with broad subround apical margin, large hair on apex of blade, outer and inner margins slightly sinuate, stem squarish, inner margin subround with a little concavity; aedeagus (Fig. 21) with phallosoma large and wide especially in proximal region, conjunctiva with a pair of semisclerotized ventral appendages, vesica thick and not much coiled, ejaculatory reservoir round, basal plate with pivot.

H. lacertosus Distant:

Pygophore (Fig. 22) broadly oval, outer margin subround anteriorly, sinuate posteriorly, gradually narrowed posteriorly, ventroposterior margin or lip broad and substraight, hair on posterior side; paramere (Fig. 23) with outer margin round and medially prominent, blade long and thick and gradually becoming narrowed posteriorly, apex with two processes, stem rectangular with inner margin slightly sinuate, hair on blade and stem; aedeagus (Fig. 24) with phallosoma very large and wide, conjunctiva with a pair of semisclerotized ventral appendages, vesica thicker with three coils, basal plate with pivot.

H. prominulus Dallas:

Pygophore (Fig. 25) oblong, outer margin subprominent and subround, gradually narrowing posteriorly and sinuate, ventroposterior margin or lip narrow, medially sinuate, ventroanterior opening triangular; paramere (Fig. 26) with outer margin medially subround with long and slender blade, apex of blade little sinuate with small acute process, outer margin substraight, inner and outer margin substraight, stem more or less squarish with inner margin concave; aedeagus (Fig. 27) with phallosoma large and wide, especially in proximal region, conjunctiva with sclerotized appendages in the ventral region, vesica thick with two coils, ejaculatory reservoir round, a pair of membranous appendages at base of vesica, basal plate with lateral appendages known as pivot.

H. signatus Walker:

Pygophore (Fig. 28) very broad anteriorly, outer margin substraight up to middle, posteriorly narrow and subround, ventroposterior margin or lip broad and subround, with hair, medially little sinuate; paramere (Fig. 29) with outer margin subround, medially subprominent, blade long and thick with equal breadth, apex with substraight margin, outer and inner margins sinuate, stem squarish with inner straight margin without any concavity, hair on apex of blade and inner of stem; aedeagus (Fig. 30) with phallosoma large and wide especially in proximal region, conjunctiva with a pair of dorsal membranous appendages which are leaf shaped and a pair of semisclerotized ventral membranous appendages, vesica thin and coiled, ejaculatory reservoir round, basal plate with pivot.

Abbreviations

B: Blade, BP: Basal Plate, C: Conjunctiva, DMA: Dorsal membranous appendages, ER: Ejaculatory Reservoir, Fig.: Figure, GP: Gonoporal Process, LSA: Lateral Sclerotized Appendages, LSA: Lateral Sclerotized Appendages, mm: Millimeters, OM: Outer margin, P: Pivot, St: Stem, T: Theca, V: Vesica, VAM: Ventroanterior Margin, VPM: Ventroposterior Margin, VSA: Ventral Sclerotized Appendages.

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Seasonal biology of a geometrid looper, *Hyposidra infixaria* (Lepidoptera: Geometridae) on tea [*Camellia sinensis* (L) O. Kuntz.]

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[2]*Department of Agricultural Entomology, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal*
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ABSTRACT: Seasonal biology of a geometrid looper, *Hyposidra infixaria* walker was studied during summer (April–May), monsoon (August September) and winter (December–February) of 2008–09 under laboratory conditions. The longest incubation period was recorded in winter (11.20 ± 0.16 days) followed by monsoon (6.35 ± 0.08 days) and summer (5.20 ± 0.06 days). Similarly, larval period was the longest in winter (32.29 ± 0.38 days) than summer (16.17 ± 0.38 days) and monsoon (16.95 ± 0.31 days). Total developmental period was the shortest during summer (31.11 ± 0.32 days) followed by monsoon (33.60 ± 0.22 days) and longest in winter (57.88 ± 0.46 days). However, longevity of adult moths did not vary with seasons and ranged between 4–6 days. Larvae and pupae developed in winter were significantly shorter in size and lighter in weight than developed during summer and monsoon. © 2011 Association for Advancement of Entomology

KEYWORDS: Seasonal biology, *Hyposidra infixaria*, looper, tea plantations

INTRODUCTION

Tea [*Camellia sinensis* (L) O. Kuntz.] is an evergreen perennial cultivated as the major plantation crop in northeast India including Dooars (26^0 44′ N to 26^0 54′ N latitude and 88^0 55′ E to 89^0 28′ E longitudes). In India, about 300 species of insects, mites and nematodes have been found to infest tea of which about 25 species are recognized as serious pests (Mukherjee and Singh, 1993). Among the different insects, leaf feeding Lepidopteron constitute a large proportion of tea pests that cause considerable damage to the crop. Out of some 200 species of caterpillars recognized

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as pests of tea in different parts of world, geometrids in India and China, tortricids in Sri Lanka and Japan, limacodids in Borneo are considered major ones (Hazarika *et al.*, 2001). Basu Majumder and Ghosh (2009) reported the occurrence of 6 species of geometrid defoliators belonging to 5 genera in the tea plantation of Dooars. Of these loopers, *Hyposidra infixaria* Wlk. (Lepidoptera: Geometridae), commonly known as black looper has become a major concern for the Dooars tea plantations and found to cause severe damage to tea bushes all-round the year. Studies on the biology of insect pests are important for taking decision on their management strategies. Earlier, Nair *et al.* (2008) and Basu Majumder and Talukder (2009) have reported the biology of this insect on tea. However, comprehensive studies on the seasonal biology of this insect pest are limited. Therefore, the present study was undertaken to examine the seasonal biology of *H. infixaria* on tea in details which would ultimately help in its management in tea plantations of Dooars.

MATERIALS AND METHODS

Seasonal biology of *Hyposidra infixaria* Wlk. was studied at the laboratory of Plant Protection unit of North Bengal Regional R&D Centre (erstwhile Nagrakata sub-station) of Tea Research Association, West Bengal, India during summer (April–May), Monsoon (August–September) and winter (December–February) of 2008–09. An initial laboratory culture of the insect was established and maintained on tea foliage from field collected loopers. Upon pupation, male and female pupae were kept in fine mesh rearing cages (12″× 10″ × 8″) for adult emergence. Freshly emerged females were found to mate with males and were observed regularly for egg laying. After Oviposition, the eggs were observed for hatching. 40 neonate larvae of *H. infixaria* that hatched on the same day were transferred individually in tender tea shoots bearing a bud and 2–3 leaves obtained from the pesticide free bushes maintained at the T.R.A experimental plot. The transfer of larvae to the shoots was done with the help of fine soft brush to avoid any injuries. Each shoot was kept in 2″ reagent bottle containing water and plugged with cotton to hold it in position. The bottles were placed individually in 15 mm diameter petri-dishes containing paper towel and a glass chimney (10″) was put on it. The top of the chimneys were covered with fine malmal cloth to prevent escape of loopers. The shoots were replaced every second day for first and second instar larvae. However, the later instars were given semi matured leaves as and when required. The same rearing procedure was followed for every time the biology studied. The number and duration of different larval instars were calculated on the basis of larval moulting. Larval moulting was checked daily at 16:00 hours during different season of rearing. Measurements of moulted head capsules were taken with the help of ocular micrometer attached to an Olympus SZX7 stereo-microscope. Larval period was determined from the date of hatching to pre pupal stage. Prepupal period was recorded as end of larval feeding until pupation. Similarly pupal period was recorded as the time between formation of pupa and adult eclosion. Measurements on length and weight of larval instars, pre-pupae and pupae were made on a sample of 20 individuals to characterize the insect with the help of standard ruler (0.25 mm

precision) and digital balance (Sartorius L2200S) respectively. Data thus collected were analyzed following ANOVA and means were separated by Duncan's Multiple Range Tests with the help of statistical software SPSS 10.0.

RESULTS

The loopers of *H. infixaria* were reared successfully under laboratory conditions during different seasons of the year on tea foliage.

Egg

Under laboratory caged condition, female moths deposited their eggs indiscriminately on the mesh wall of cage, joints of wooden bits, or some times on the paper towel in clusters. Each egg was 0.79±0.01 (0.72–0.85) mm in length and 0.55±0.01 (0.43–0.62) mm in diameter (n = 20), bluish green in colour with both ends rounded. Before hatching they assumed dark colour. On an average, irrespective of seasons, female moths laid 641.00 ± 31.34 (552–728) eggs and about 97.4% of the eggs hatched successfully in the laboratory. Incubation period varied significantly among the seasons. Overall, incubation period of the insect ranged between 5–12 days and was the longest in winter (11.20 ± 0.16 days) followed by monsoon (6.35 ± 0.08 days) and summer (5.20 ± 0.06 days).

Larva

The larvae of *H. infixaria* were found to develop through 5 or 6 larval instars within the same population during the entire period of study. Since majority of the larvae (>50%) larvae developed through 5 instars, larval description and seasonal biology is reported here based on the observations of 5 instars larvae.

It was observed that total larval period did not differ between summer and monsoon but varied significantly in winter. Total larval period ranged from 14–19 days in summer and monsoon but 29–35 days in winter with mean larval duration of 16.17 ± 0.38 days in summer, 16.95 ± 0.31 days in monsoon and 32.29 ± 0.38 days in winter. Irrespective of seasons, mean duration of second instar was the shortest (2.17 ± 0.09 days, 2.25 ± 0.10 days and 4.59 ± 0.12 days in summer, monsoon and winter respectively) whereas fifth instar took longest time to develop (4.94 ± 0.24 days, 5.20 ± 0.21 days and 8.00 ± 0.26 days in summer, monsoon and winter respectively) (Table 1).

The early instar loopers were chocolate brown to black in colour with 6 white cross bands on the body. These bands are not continuous rather made up of small white spots and located dorso-laterally but absent ventrally. In addition to the thoracic legs, two pairs of prolegs were present on 6th and10th abdominal segments. Setae sparsely present on head and body. The important change in later instar loopers was the pattern of body colour and gradual prominence of a broad lateroventral black stripe originating from the metathoracic legs of both sides that extends up to the 1st abdominal segment. At these stages, the transverse white bands though present but became fainter gradually

TABLE 1. Seasonal biology of a geometrid looper, *Hyposidra infixaria* Wlk. on tea under laboratory condition

Stage	Mean duration (days) ± SE during		
	April to May	August to September	December to February
	Max. temp. (^{0}C) 29.8± 1.2	Max. temp. (^{0}C) 28.2 ± 1.9	Max. temp. (^{0}C) 21.6± 1.8
	Min. temp (^{0}C) 26.0 ± 1.3	Min. temp (^{0}C) 25.5± 0.7	Min. temp (^{0}C) 17.9 ± 1.6
	R.H. (%) 75.4± 8.3	R.H. (%) 89.3± 6.3	R.H. (%) 76.9 ± 10.0
Egg	5.00c*	6.00b	10.00a
Larval instars			
I	3.67 ± 0.11b (3–4)	3.90 ± 0.07b (3–4)	8.06± 0.19a (7–9)
II	2.17 ± 0.09b (2–3)	2.25 ± 0.10b (2–3)	4.59 ± 0.12a (4–5)
III	2.61 ± 0.12b (2–3)	2.80 ± 0.10b (2–3)	5.29 ± 0.17a (4–6)
IV	2.78 ± 0.13b (2–4)	2.80 ± 0.09b (2–3)	6.35 ± 0.23a (6–8)
V	4.94 ± 0.24b (3–7)	5.20 ± 0.21b (4–7)	8.00 ± 0.26a (7–10)
Larva	16.17 ± 0.38b (14–19)	16.95 ± 0.31b (14–19)	32.29 ± 0.38a (29–35)
Pre-pupa	2.11 ± 0.08b (2–3)	2.10 ± 0.07b (2–3)	3.76 ± 0.14a (3–4)
Pupa	7.83 ± 0.17c (7–9)	8.55 ± 0.22b (7–10)	11.82 ± 0.36a (10–15)
Total duration	31.11 ± 0.32c (29–33)	33.60 ± 0.22b (32–35)	57.88 ± 0.46a (55–61)
Moths			
Male	4.91 ± 0.28a (4–6)	4.42 ± 0.25a (4–6)	4.36± 0.21a (4–6)
Female	5.29 ± 0.29a (4–6)	4.88 ± 0.30a (4–6)	4.80 ±0.37a (4–6)

Figures in parentheses denote ranges. *Studies were conducted with those neonates that hatch on same day from a single egg mass. Means followed by the same letter in row does not differ at 5% level of significance by DMRT.

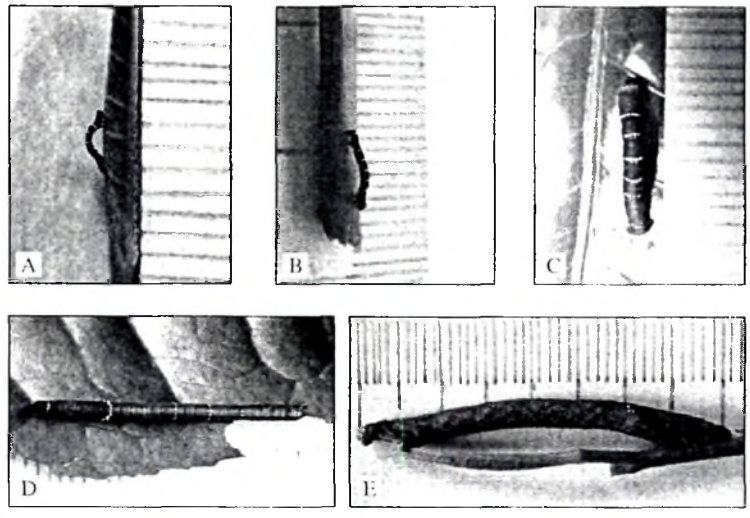
on each moulting except the one on 1st abdominal segment. Body became stout and cylindrical with smooth surface. Head region was shiny, slightly smaller than body, smooth and faintly marked with mottlings. Legs were light orange in colour, prolegs were stout (Fig. 1).

Prepupa:

After completion of feeding, the last instar larvae entered into a quiescent stage. The body size gradually reduced due to shrinkage. Initially, prepupae were dull green in colour with dark black head and a prominent ventral line. Prepupal period also did not differ between summer and monsoon but significantly higher in winter. Mean prepupal period in summer, monsoon and winter was 2.11 ± 0.08 days, 2.11 ± 0.07 days and 3.76 ± 0.14 days respectively.

Pupa:

Soon after the larval-pupal moult, the prepupa developed into pupa. Unlike prepupal period, mean pupal period varied significantly among the seasons. It was longest in winter (11.82 ± 0.36 days) followed by monsoon (8.55 ± 0.22 days) while shortest in summer (7.83 ± 0.17 days). Pupation mostly occurred on paper towel of the Petri

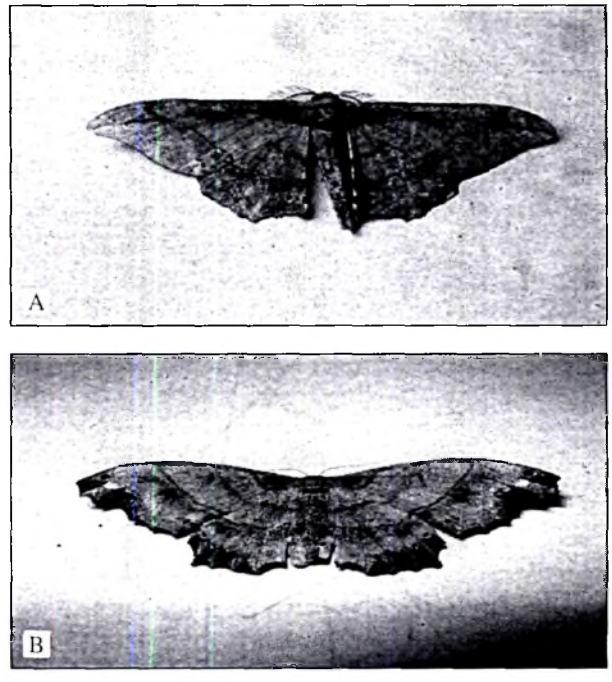


FIGURES 1. Different larval stages of *Hyposidra infixaria* (individuals with 5 instars) A: 1st instar. B: 2nd instar. C: 3rd instar. D: 4th instar. E: 5th instar. Scale: 1 mm.

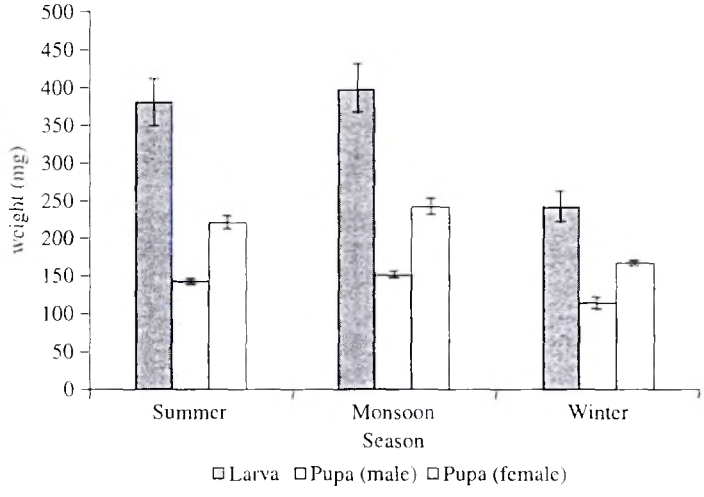
dishes or sometimes on the region where tea shoots were plugged with cotton in the bottle. The pupa was initially green in colour but soon changed into dark brown in colour. The terminal segments of the pupa tapered sharply to a distinctive cremester which bear a bifurcated process that curved upwards. The eyes were prominent, dark and protruding. The cephalic end was evenly rounded. The body surface of the pupa was smooth and glistening. Female pupa was larger than male pupa.

Significant variation was also recorded in the mean developmental period (Oviposition to adult eclosion) of *H. infixaria* when reared on tea foliage during different seasons. Loopers took shortest time to develop during summer (31.11 ± 0.32 days), followed by monsoon (33.60 ± 0.22 days) whereas developmental period was longest in winter (57.88 ± 0.46 days). However, longevity of adult moths did not vary with seasons. Both male and female moths lived for 4–6 days in all the seasons. The wing colour of the moths ranged from straw to grayish or rich brown. Males had bi-pectinate antennae, smaller than females with a small tuft of hairs at the tip of their abdomen. In many male moths a longitudinal subcostal line was present in that colour (Fig. 2).

Like duration, length and weight of different stages of the insect were found to vary among the seasons. Mean larval and pupal length was significantly shorter in winter than other two seasons (Table 2). Similarly, larvae and pupae developed during winter were significantly lighter in weight than summer and monsoon (Fig. 3). Larval weight ranged from 210–660 mg in summer and monsoon but 160–480 mg in winter. Similarly, male and female pupae weighed around 70–160 mg and 150–190 mg respectively in winter whereas in other two seasons pupal weight was ranged from 120–180 mg (male pupae) and 170–290 mg (female pupae). Mean wing expanse of



FIGURES 2. Moths of *Hyposidra infixaria*, A: Male moth. B: Female moth



FIGURES 3. Comparative weight (mean ±SE) of larvae and pupae of *H. infixaria* fed on tea in different seasons

TABLE 2. Comparative length (mean ± SE) of different stages of *H. infixaria* fed on tea in different seasons

	April to May (Summer)	August to September (monsoon)	December to February (winter)
Larval length (mm)	44.06 ± 1.17a (35–52)	45.00 ± 1.18a (35–52)	38.39 ± 0.83b (30–45)
Pupal length (mm)			
Male	13.82 ± 0.26a (13–15)	14.09 ± 0.25a (13–15)	13.00 ± 0.19b (12–14)
Female	17.00 ± 0.27a (16–18)	17.09 ± 0.25a (16–18)	15.82 ± 0.23b (15–17)
Wing expanse (mm)			
Male	35.50 ± 0.34a (33–37)	35.00 ± 0.39a (32–37)	33.83 ± 0.46b (32–37)
Female	47.38 ± 0.71a (45–50)	47.00 ± 0.78a (44–50)	46.38 ± 0.63a (45–50)

Figures in parentheses denote ranges. Means followed by the same letter in row does not differ at 5% level of significance by DMRT.

male moths developed in winter (33.83 mm) was significantly shorter than summer (35.00 mm) and monsoon (35.50 mm). However, no such variation was observed in case of female moths. Irrespective of seasons, the wing expanse of female moths ranged from 44–50 mm.

Discussion

This study showed that under laboratory conditions, the mean number of eggs laid by the female moth of *H. infixaria* was 641 irrespective of different seasons which were lower than the findings of Nair *et al.* (2008) who recorded the average fecundity of the insect was 761.1. This reproductive difference of *H. infixaria* females could be due to the variations in the environmental conditions or genetic or other factors which triggered more egg laying in the later study and was not examined in the present study. However, the incubation period under present study during monsoon confirmed the results of Nair *et al.* (2008) where they reported that at 28 ± 1°C temperatures and 88 ± 2% R.h., eggs of *H. infixaria* took 6–7 days to hatch. In general, incubation period was shorter in summer and longer during winter. In another study, Ananthanarayanan and Vengupal (1953) recorded the hatching of *Hyposidra successaria* eggs within 5–6 days in summer.

According to the present study, number of larval instars varied within the same population of *H. infixaria*. In all the seasons, majority of the loopers developed through 5 instars while 6 instar individuals were there. Such variation in number of larval instars within the same population is common in many lepidopterans as earlier reported by Casimero *et al.* (2000) in *Helicoverpa armigera*, Berndt *et al.* (2004) in endemic geometrid *Pseudocoremia suavis*, Rodríguez-Loeches and Barro (2008) in arctiid moth, *Phoenicoprocta capistrata*. However, Nair *et al.* (2008) reported only six larval instars in the loopers of *H. infixaria*.

Like present study, similar kind of seasonal variation in larval period was recorded by Bardoloi and Hazarika (1992) in *Antheraea assama*, a multivoltine commercial silk producing insect of Assam, India where larval period was longest during

winter (December–February) and shortest during summer (May–July). In another study, Ananthanarayanan and Vengupal (1953) observed that larval, pupal and total developmental period of *H. successaria* ranged from 15–21 days, 7–9 days and 29–31 days during summer and monsoon season respectively. Larval duration during summer-monsoon as observed by Nair *et al.* (2008) was slightly longer than the present study which again might be due to one additional instar of *H. infixaria* in their studies. The other probable causes might be differences in rearing conditions and quality of tea foliage fed to the loopers in both the studies. However, Adult longevity of *H. infixaria* determined in the present work was similar to that reported by Nair *et al.* (2008).

One of the important findings of the present study was the variation in lengths and weights of different stages of *H. infixaria* among different seasons. Larval and pupal lengths were significantly higher in summer-monsoon than winter. Similarly, fresh weight of larvae and pupae developed during summer-monsoon were heavier than those developed in winter. However, in the present study, no specific experiment was carried out to identify and quantify the factors influencing this seasonal variation but temperature and nutritional quality of tea leaves could be presumed as the two major factors attributing to this variation. It was established from the studies of Erturk *et al.* (2010) and Kottura *et al.* (2010) that biochemical parameters of green shoots of tea varied with seasons. Therefore, chances of variation in body lengths and weights of larvae and pupae of *H. infixaria* were more likely due to varied nutritional quality of tea shoots fed to the insect in different seasons. A similar kind of observation was reported by Rodrigues and Moreira (2004) in *Heliconius erato phyllis* (Lepidoptera: Nymphalidae) where seasonal variation of adult size at a given place was primarily determined by the availability and quality of host plant species used by the larval stage rather than the temperature during larval development. Overall, the study provided important information on the seasonal biology of looper, *H. infixaria* and as such will be helpful for managing the pest in tea plantations of Northeast India including Dooars.

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Studies on genetic divergence in tropical bivoltine silkworms, *Bombyx mori* L. (Lepidoptera: Bombycidae)

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ABSTRACT: Seventeen bivoltine silkworm breeds were examined for the genetic divergence using Mahalanobis D^2 statistic and canonical root analysis. The breeds were grouped into six clusters indicating the presence of distinct diversity that was further confirmed by canonical root analysis. The bivoltine breeds CSR-26, NP-2, KSO-1, CSR-4 and NB_4D_2 were grouped in cluster I, KPGB, APS-4, APS-5, CSR-27, APS-8 and KPGA in cluster II, CSR-6 in cluster III, SH-6 in cluster IV, CC-1, CSR-2 and NB_7 in cluster V and NB_{18} in cluster VI. Highest genetic divergence was found between cluster II and VI (5.67), followed by cluster I and VI (5.46), V and VI (5.08) and III and VI (4.24). Cocoon yield (41.18%), silk productivity (25.74%), larval duration (19.12%), pupal weight (5.15%) and cocoon weight (4.41%) largely contributed to the divergence. © 2011 Association for Advancement of Entomology

KEYWORDS: Silkworm, *Bombyx mori* L., bivoltine, genetic divergence

INTRODUCTION

Global silk production is centered around tropical countries and India is one among them ranking second next only to China. In India bulk of the silk is produced by using hybrid silkworms i.e. multivoltine × bivoltine which qualitatively does not fit into international standards. High quality international grade silk can be produced only by using bivoltine silkworms (Jaiswal and Goel, 2003). This has necessitated for the development of bivoltine silkworm breeds *viz*., NB_{18}, NB_7, NB_4D_2, KA and CC-1 by various sericulture research institutes and hybrids were evolved by

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utilizing these breeds. However, these hybrids couldn't adapt to the climatic conditions of tropical region due to the temperate origin of the bivoltine parents used for developing new breeds in India. In silkworm (*Bombyx mori*) the silk yield and yield attributes largely depends on a wide range of environmental conditions (Ren *et al.*, 1988). Bivoltine silkworm breeding over a decade has resulted in evolution of new robust bivoltine breeds suitable to tropical environment. But, for commercial rearing bivoltine hybrids that are productive and adaptable to the local climatic condition is essential. In the direction selection of parents is often challenging to a breeder to achieve a desired heterotic combinations. Determination of genetic divergence among the parents may facilitate the selection of parents for developing a desired hybrid combination. Manifestation of heterosis in F1's is reported to be related to the optimum level of genetic divergence between parents (Arunachalam *et al.*, 1984; Jinfei and Wang, 1983). It has been felt necessary to assess the genetic divergence among the bivoltine silkworms to select the parents for developing the hybrids suitable to climatic conditions of non traditional sericulture region of northern Karnataka state, India.

MATERIALS AND METHODS

Seventeen bivoltine silkworm pure breeds were used for the study (Table 1). The breeds were reared twice during August to November, 2008 at Dharwad, India that is located between 15° 25′ N latitude and 70° 26′ N longitude at an altitude of 658 m above mean sea level. The place is classified as transitional agricultural zone-VIII with the temperature and relative humidity varying between 13.30°C to 36.50°C and 57.50% to 93.20%, respectively and receives 776 mm rainfall per annum. Three replications were maintained for each breed with a disease free laying (500 silkworms) per replication. Standard bivoltine silkworm rearing technology (Dandin *et al.*, 2000) was followed in the study. Observations were made on fifteen economic traits (Table 2). The mean values were used for analysis of variance and to estimate D^2 statistic (Mahalanobis, 1936) and genetic distance among the genotypes. Following Tocher's method as given in Rao (1952) clusters were made and confirmed by canonical root analysis as described by Singh and Choudhary (1985).

RESULTS

Study revealed a significant difference among the means of the populations of the breeds and for all the traits except total larval duration, indicating the presence of variability among the breeds (Table 2).

The genetic divergence among the breeds as measured by using Mahalanobis D^2 statistic has led into six distinct clusters (Table 3). The breeds, CSR-26, NP-2, KSO-1, CSR-4 and NB_4D_2 were grouped in cluster I. Cluster II comprised of KPGB, APS-4, APS-5, CSR-27, APS-8 and KPGA. CSR-6 and SH-6 were grouped in cluster III and IV, respectively. Cluster V comprised of CC-1, CSR-2 and NB_7 and NB_{18} was grouped in cluster VI. The pattern thus reveals that there is no clear cut relationship between genetic diversity and the geographical origin of the breeds.

TABLE 1. Characteristics of the tropical bivoltine silkworm breeds used in the study

Breeds	Parentage	Evolved at	Larval marking	Cocoon shape	Cocoon colour	Cocoon grains
SH-6	Shungetsu x Hosho	RSRS, Dehradun	Marked, bluish white	Oval	Bright white	Medium
CC-1	(NB7 x NB-1) x (KA x SPC-1)	CSR&TI, Mysore	Plain, bluish white	Oval	Bright white	Medium
KPGA	(N122 x C110) x (N124 x C124)	RSRS, Kalimpong	Plain, bluish	Oval	Bright white	Medium
KPGB	N501 x C502	RSRS, Kalimpong	Plain, bluish	Oval	Bright white	Medium
NB-	Kinshu x Showa	CSR&TI, Mysore	Plain, bluish white	Oval	Bright white	Medium
CSR-2	Shunrei x Shogetsu	CSR&TI, Mysore	Plain, bluish white	Oval	Bright white	Fine to medium
CSR-27	Thaihei x Choan	CSR&TI, Mysore	Plain	Oval	Bright white	Fine to medium
KSO-1	NB_4D_2 x NP1	KSSR&DI, Bangalore	Plain	Oval White	Medium	
APS-5	Hemavathy (line separation)	APSSR&DI, Hindupur	Plain, bluish white	Oval	White	Medium
NB_4D_2	(Kokox Seihaku) x (N124 x C124)	CSR&TI, Mysore	Plain, bluish white	Dumbbell	Bright white	Medium
NB_{18}	(Kokox Seihaku) x (N124 x C124)	CSR&TI, Mysore	Plain, bluish white	Dumbbell	Bright white	Medium
CSR-4	(BN18 x BCS25) x NB_4D_2	CSR&TI, Mysore	Plain, bluish white	Dumbbell	Bright white	Fine to medium
CSR-6	Shunrei x Shogetsu	CSR&TI, Mysore	Marked, reddish tinge	Dumbbell	Dull white	Fine to medium
CSR-26	C135 x NB4	CSR&TI, Mysore	Marked, dull white	Dumbbell	White	Fine to medium
NP-2	PM x N.P.	KSSR&DI, Bangalore	Plain	Dumbbell	White	Medium
APS-4	TR (line separation)	APSSR&DI, Hindupur	Plain, bluish white	Medium dumbbell	White	Medium
APS-8	Swarna (line separation)	APSSR&DI, Hindupur	Plain, bluish white	Medium dumbbell	White	Medium

TABLE 2. Mean performance of tropical bivoltine silkworm breeds for quantitative traits

Breeds	Fifth instar larval duration (h)	Total larval duration (h)	Chawki larval weight (g/10 larvae)	Mature larval weight (g/10 larvae)	Silk productivity (cg/day)	Survival (%)	Pupation (%)
SH-6	190^{abc}	625	2.200^{a-d}	44.965^{bc}	3.980^{abc}	$85.00\ (67.26)^{a}$	$96.33\ (78.96)^{a}$
NB_7	183^{a}	625	1.885^{cd}	41.015^{c}	3.755^{c}	$77.33\ (61.58)^{abc}$	$92.66\ (74.35)^{bcd}$
KPGA	199^{a-d}	649	2.285^{a-d}	43.785^{bc}	4.065^{abc}	$82.16\ (65.03)^{abc}$	$96.00\ (78.72)^{ab}$
KPGB	200^{a-d}	650	1.860^{d}	42.335^{c}	3.315^{c}	$75.33\ (60.26)^{bc}$	$95.00\ (77.14)^{a-d}$
CC-1	184^{a}	628	2.215^{a-d}	45.785^{abc}	4.335^{abc}	$77.16\ (61.49)^{abc}$	$97.00\ (80.04)^{a}$
CSR-2	188^{ab}	641	2.495^{ab}	49.805^{ab}	5.230^{a}	$82.33\ (65.18)^{abc}$	$91.83\ (73.41)^{d}$
CSR-27	219^{d}	661	2.290^{a-d}	43.220^{bc}	3.985^{abc}	$80.67\ (63.92)^{abc}$	$95.00\ (77.19)^{a-d}$
KSO-1	206^{a-d}	622	2.445^{abc}	52.235^{a}	5.160^{ab}	$84.50\ (66.82)^{ab}$	$95.00\ (77.19)^{a-d}$
APS-5	211^{bcd}	649	2.145^{a-d}	46.785^{abc}	3.935^{abc}	$81.33\ (64.42)^{abc}$	$92.17\ (73.75)^{cd}$
NB_4D_2	217^{d}	627	2.215^{a-d}	44.230^{bc}	3.825^{bc}	$84.83\ (67.09)^{a}$	$95.50\ (77.81)^{abc}$
NB_{18}	183^{a}	601	1.865^{d}	40.805^{c}	4.185^{abc}	$78.33\ (62.26)^{abc}$	$94.16\ (76.03)^{a-d}$
CSR-4	218^{d}	636	2.610^{a}	45.525^{abc}	4.065^{abc}	$79.17\ (62.86)^{abc}$	$91.83\ (73.40)^{d}$
CSR-6	210^{bcd}	617	2.125^{a-d}	40.165^{c}	4.015^{abc}	$75.83\ (60.56)^{abc}$	$95.50\ (77.81)^{a-d}$
CSR-26	210^{bcd}	616	2.370^{a-d}	44.465^{bc}	4.305^{abc}	$74.83\ (59.91)^{c}$	$95.84\ (78.23)^{abc}$
APS-4	220^{d}	653	1.995^{bcd}	40.455^{c}	3.165^{c}	$74.67\ (59.82)^{c}$	$95.00\ (77.10)^{a-d}$
APS-8	211^{bcd}	660	2.275^{a-d}	42.865^{bc}	4.045^{abc}	$74.66\ (59.79)^{c}$	$96.33\ (78.96)^{a}$
NP-2	214^{cd}	623	2.320^{a-d}	50.300^{ab}	4.265^{abc}	$80.50\ (63.86)^{abc}$	$96.33\ (78.97)^{a}$
F Test	*	NS	*	**	**	**	**
S.Em ±	8.606	-	0.119	1.559	0.280	1.910	0.717

TABLE 2. contd.

Breeds	Pupal weight (g/10 pupae)	Fecundity (No.)	Cocoon weight (g/10 cocoons)	Cocoon shell weight (g/10 shells)	Cocoon shell ratio (%)	Cocoon yield/25 dfls	Cocoon filament length (m)	Denier
SH-6	15.555^{abc}	513^{cd}	18.825^{bcd}	3.155^{bcd}	16.84 (24.22)^{bc}	11.345^{de}	863.17^{cde}	2.53^{ab}
NB_7	13.915^{bcd}	549^{abc}	16.910^{cd}	2.885^{cd}	17.01 (24.36)^{bc}	11.730^{cd}	816.33^{de}	2.08^{a}
KPGA	17.145^{a}	543^{abc}	20.610^{ab}	3.370^{bcd}	16.35 (23.84)^{c}	11.820^{cd}	952.67^{a-d}	2.69^{ab}
KPGB	14.145^{bcd}	592^{abc}	16.940^{cd}	2.760^{d}	16.33 (23.81)^{c}	11.950^{cd}	805.50^{de}	2.74^{ab}
CC-1	16.235^{abc}	501^{d}	19.650^{abc}	3.320^{bcd}	16.88 (24.26)^{bc}	14.140^{ab}	837.17^{cde}	2.46^{ab}
CSR-2	16.245^{abc}	577^{abc}	20.370^{abc}	4.050^{ab}	19.90 (26.49)^{ab}	14.675^{a}	1066.17^{ab}	2.73^{ab}
CSR-27	14.470^{a-d}	529^{bcd}	18.550^{bcd}	3.565^{a-d}	19.74 (26.37)^{ab}	12.090^{cd}	1011.84^{abc}	2.63^{ab}
KSO-1	17.325^{a}	551^{abc}	22.925^{a}	4.455^{a}	19.32 (26.07)^{abc}	13.795^{ab}	1121.50^{a}	3.00^{b}
APS-5	14.755^{a-d}	531^{a-d}	18.425^{bcd}	3.440^{bcd}	18.68 (25.61)^{abc}	12.935^{bc}	730.00^{e}	2.04^{a}
NB_3D_2	16.010^{abc}	625^{a}	19.640^{abc}	3.445^{bcd}	17.61 (24.81)^{abc}	11.710^{cd}	880.83^{cde}	2.66^{ab}
NB_{18}	14.545^{a-d}	618^{ab}	17.915^{bcd}	3.195^{bcd}	17.85 (24.99)^{abc}	9.365^{d}	730.00^{e}	2.81^{ab}
CSR-4	14.940^{a-d}	531^{a-d}	18.740^{bcd}	3.690^{a-d}	19.66 (26.31)^{ab}	12.455^{cd}	1096.84^{a}	2.63^{ab}
CSR-6	13.630^{cd}	564^{abc}	17.255^{bcd}	3.525^{bcd}	20.36 (26.82)^{a}	10.450^{cd}	1114.17^{a}	2.49^{ab}
CSR-26	15.500^{abc}	567^{abc}	19.235^{bc}	3.755^{abc}	19.72 (26.37)^{ab}	13.955^{ab}	968.84^{a-d}	2.93^{ab}
APS-4	12.440^{d}	554^{abc}	15.710^{d}	2.900^{cd}	18.46 (25.44)^{abc}	10.480^{cd}	894.17^{b-e}	2.97^{b}
APS-8	14.970^{a-d}	565^{abc}	18.570^{bcd}	3.540^{a-d}	19.08 (25.91)^{abc}	13.675^{ab}	895.67^{b-e}	2.48^{ab}
NP-2	16.660^{ab}	550^{abc}	20.570^{ab}	3.800^{abc}	18.22 (25.27)^{abc}	14.835^{a}	912.20^{b-e}	2.94^{b}
F Test	**	**	**	**	**	**	**	*
S.Em±	0.617	19.530	0.737	0.197	0.673	0.273	39.153	0.170

Figures in parentheses are angular transformed values.

*significant at 5 per cent ** significant at 1 per cent.

In vertical columns, means followed by similar letters are not different statistically by Duncan's Multiple Range Test (DMRT) at 5% probability *significant at 5 per cent ** significant at 1 per cent.

TABLE 3. Distribution of bivoltine silkworm breeds in different clusters

Cluster	Breeds
I	CSR-26, NP-2, KSO-1, CSR-4 and NB_4D_2
II	KPGB, APS-4, APS-5, CSR-27, APS-8 and KPGA
III	CSR-6
IV	SH-6
V	CC-1, CSR-2 and NB_7
VI	NB_{18}

TABLE 4. Intra and inter cluster D^2 and $\sqrt{D^2}$ (in parentheses) values of six clusters of bivoltine silkworm breeds

Clusters	I	II	III	IV	V	VI
I	13.50 (3.67)					
		18.47 (4.29)	16.76 (4.11)	18.32 (4.28)	19.05 (4.36)	29.83 (5.46)
II		12.34 (3.51)	19.82 (4.45)	19.38 (4.40)	19.14 (4.37)	32.18 (5.67)
III			0.00	10.73 (3.27)	19.66 (4.43)	18.02 (4.24)
IV				0.00	16.86 (4.10)	15.91 (3.98)
V					15.77 (3.97)	25.87 (5.08)
VI						0.00

Perusal of the intra-cluster values in Table 4 revealed a narrow difference among the intra clusters values of cluster I, II and V. On the basis of cluster distance, highest genetic divergence was found between cluster II and VI (5.67), followed by cluster I and VI (5.46), V and VI (5.08) and III and VI (4.24). While, minimum divergence was noticed between IV and VI (3.98).

The most important traits contributing towards the divergence were cocoon yield (41.18%), silk productivity (25.74%), larval duration (19.12%), pupal weight (5.15%) and cocoon weight (4.41%) (Table 5).

In the present study it would appear from the canonical analysis that the five roots with a value of 1334.95, 729.29, 461.17, 237.74 and 66.89 and accounted for 45.21, 24.69, 15.61, 8.05 and 2.26 per cent variability (Table 6) and a total of 95.84 per cent of variation was accounted. An examination of five vectors confirmed the potent role of larval duration, cocoon yield, silk productivity, pupal weight and cocoon weight in differentiating among the breeds as seen from the magnitude of the coefficients in canonical root analysis

TABLE 5. Percentage contribution of each quantitative traits to the divergence

Characters	Contribution (%)
Fifth instar larval duration	0.00
Total larval duration	19.12
Chawki larval weight	0.00
Mature larval weight	0.74
Silk productivity	25.74
Survival	0.00
Pupation	0.74
Pupal weight	5.15
Cocoon weight	4.41
Cocoon shell weight	0.00
Cocoon shell ratio	0.00
Cocoon yield	41.18
Cocoon filament length	1.47
Denier	1.47
Fecundity	0.00

TABLE 6. Values of the coefficients of the canonical root analysis

Characters	Vector 1	Vector 2	Vector 3	Vector 4	Vector 5
Fifth instar larval duration	0.04050	0.00585	−0.03391	−0.01318	−0.05019
Total larval duration	0.43657	0.49063	0.09344	0.43362	0.09377
Chawki larval weight	0.21728	0.02106	−0.04369	0.30097	0.02816
Mature larval weight	0.14605	−0.16382	0.00936	0.27488	−0.31653
Silk productivity	−0.36658	−0.16678	0.75704	0.20049	0.17405
Survival	0.05357	0.04307	−0.13600	0.27789	−0.30342
Pupation	0.22816	−0.00720	−0.12457	−0.21042	0.55527
Pupal weight	0.07646	−0.38820	−0.05723	0.41426	−0.07626
Fecundity	−0.03837	−0.11703	−0.13652	−0.00626	−0.17543
Cocoon weight	−0.14830	−0.47587	−0.14792	0.04758	−0.13334
Cocoon shell weight	0.14198	−0.09682	−0.08018	0.15378	−0.07874
Cocoon shell ratio	0.02203	−0.03796	−0.07473	0.01442	0.15392
Cocoon yield	0.69429	−0.39782	0.33237	−0.39122	−0.13239
Cocoon filament length	−0.07875	−0.24676	−0.46098	0.03116	0.28960
Denier	0.10829	−0.28534	0.04707	0.36354	0.52093
Root	1334.95	729.29	461.17	237.74	66.89
%Variation	45.21	24.69	15.61	8.05	2.26
Cumulative variation	45.21	69.91	85.53	93.58	95.84

DISCUSSION

Genetic divergence analysis is often used to identify suitable parents for realizing heterosis and recombination in breeding programme. Mahalanobis D^2 statistic has been followed by several workers to measure the genetic distance among the breeding

materials and to identify the characters responsible for the divergence (Rao *et al.*, 1981; Jolly *et al.*, 1989; Razdan *et al.*, 1994; Arunachalam *et al.*, 1984).

In the present study the seventeen bivoltine silkworm breeds were found to form six distinct clusters indicating the presence of distinct diversity among them. These breeds can therefore be successfully used in the breeding programme to develop F_1 hybrids that will yield higher magnitude of heterosis. It was also revealed from the study that the breeds derived from the same parents were included in different clusters showing the variation among the breeds. On the other hand the breeds derived from different sources at different regions were included in the same clusters showing affinity between advanced sister lines. Hence, the genetic diversity is not synonymous with geographic diversity. Several workers have also reported that there is no relationship between silkworm breeds from diverse eco-geographic origins.

Selecting the breeds from the clusters having higher inter- cluster distance for hybridization, one can also think of selecting the parents based on the percent contribution of each characters towards the divergence. In the present study cocoon yield, silk productivity, larval duration, pupal weight and cocoon weight have contributed more to the divergence. Jolly *et al.* (1989) and Razdan *et al.* (1994) also observed that cocoon yield, larval duration and cocoon weight have contributed more towards the divergence. It is also important to consider these characters for selecting the parents. However, selection of parents based on the single complex character like cocoon yield may not show transgressive segregates for yield potential. To obtain higher heterosis the parent should be discriminated with respect to yield contributing characters and not yield itself.

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Immunocytochemical localization of glucagon, somatostatin and adipokinetic hormone-like immunoreactive secretory neurons in Castor semilooper *Achaea janata* Linn. (Lepidoptera: Noctuidae)

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ABSTRACT: Antisera against Glucagon, Somatostatin and Adipokinetic hormone (AKH) showed immuno positive reactions to selected Neurosecretory cells (NSCs) in the brain of *Achaea janata* caterpillars. The median NSCs of the brain showed somatostatin-positive immunoreactivity with polyclonals to human somatostatin. Out of the eight paraldehyde fuschin (PF)-positive MNSCs in the brain, 4–5 showed the specific somatostatin-like immunoreactivity. Another group of somatostatin-positive neurons also could be localised outside pars intercerebralis region. Immunoreactive neurons from this extending to the corpus cardiacum through the axons suggest that the material could indeed be involved in the process of neurosecretion. Human glucagon-like neurons also could be localised in certain secretory neurons on either side of pars intercerebralis region. C-terminal specific (Serial No. 241) and N-terminal specific (Serial No. 433) AKH antisera when used, positive reaction could be obtained only with AKH-241 antiserum. The studies provide additional information on the evolutionary significance and the correlation between endocrine mechanisms in vertebrates and insects. © 2011 Association for Advancement of Entomology

KEYWORDS: *Achaea janata*, Glucagon, Somatostatin, Adipokinetic hormone, Insect neuropeptides, Immunoreactive neurons

INTRODUCTION

Much of the evidence for vertebrate endocrine factors in invertebrates are immuno chemical. A number of peptides have been shown to be present in insects that share

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immunochemical similarities with vertebrate peptides. Mapping of immunoreactivity in insect nervous systems with vertebrate-hormone antibodies (Duve and Thorpe, 1982; Verhaert *et al.*, 1984; Veenstra *et al.*, 1985; Kramer, 1985) surprisingly implicate the widespread cross reactivity between vertebrate endocrine principles and insect peptidergic factors. Of the different types of chemical messengers known in the nervous system of metazoans, neuropeptides are the most numerous and diverse (Eckert and Predel, 2000; Nassel, 2000, 2009). Neuro peptides represent the largest single class of signal compounds and are involved in the regulation of development, growth reproduction, metabolism and behaviours of insects (Alstein and Nassel, 2010). From the phylogentic point of view, it is of interest that substances antigenically related to vertebrate neuropeptides occur in the neuroendocrine system of insects and vice versa.

For many years, it has been known that the regulation of carbohydrate metabolism in vertebrates involves a multitude of hormones, enzymes, metabolites and ions (Unger *et al.*, 1978). Some of the hormones include insulin, glucagon, somatotropin and somatostatin, adrenal glucocorticoids and epinephrine. The first two hormones are the primary determinants necessary for adaptive carbohydrate changes in the vertebrate animal to respond to nutritional and environmental demands. The chemical nature of these factors which regulate carbohydrate metabolism, identified in several species of insects are shown to be similar chemically, physically and immunologically to vertebrate insulin and glucagon (Tager *et al.*, 1976; Ishay *et al.*, 1976).

While insulin serves as a hormone of fuel storage, glucagon serves as a hormone of fuel mobilization. Glucagon-immunoreactive cells were discovered in the brain of *Eristalis aeneus* (El-Salhy *et al.*, 1980), in *Manduca sexta* (El-Salhy *et al.*, 1983), in the CC-CA complexes of *M. sexta* (Tager *et al.*, 1976) and in the midgut of the cockroach *P. americana* (Iwanaga *et al.*, 1981). Raina *et al.* (1995) isolated a novel neuropeptide from the corpora cardiaca of cicadas having adipokinetic and hypertrehalosemic activities.

In both the hoverfly, *E. aeneus* and locust *L. migratoria*, somatostatin immunoreactive cells are widely distributed, being found in the brain (MNSCs), nervous part of CC and venrtal ganglia from which bundles of immunoreactive nerve fibres extend to various directions (El-Salhy *et al.*, 1980). El-Salhy *et al.* (1983) localised somatostatin-like neuropeptides in the nervous tissues of *M.sexta*. The occurence of AKH and RPCH-like neuropeptides in the CNS of different invertebrate species other than insect was investigated by Schooneveld *et al.* (1987a) with polyclonal antisera to N and C-terminal regions of AKH. The insect AKHs are a large family of peptide hormones that are involved in the mobilization of sugar and lipids from the insect fat body during energy requiring activities such as flight and locomotion and thus contribute to sugar homeostasis (Staubli *et al.*, 2002). The insect AKHs are multifunctioned (Kodrik *et al.*, 2010; Ajayakumar and Gokuldas, 2011). In view of the above facts, the present study in *A. janata* is an attempt to see the occurrence of any of mammalian insulin, glucagon and somatostatin-like peptides in the brain. AKHs are also a metabolically important hormone and studies on this were also undertaken.

MATERIALS AND METHODS

Both sexes of fifth instar larvae of *A. janata*, 140–48 hours after emergence isolated from the stock colony maintained in the laboratory under controlled conditions were used for the studies.

Tissue preparation

The caterpillars were anaesthetised and the brain, CC-CA complex were dissected out under ice-cold insect ringer, fixed in aqueous Bouin's fluid and embedded in paraplast. Sections of 5–7 thickness were cut using a rotary microtome (Spencer 820). The sections were spread and dried on glass slides.

Staining of Neurosecretory cells

For staining NSCs of brain, performic and victoria blue (PAVB) method (Dogra and Tanden, 1964), paraldehyde fuchsin (PF) method (Cameron and Steele, 1959) and Gomori's chrome alum haematoxylin phloxin (CHP) method (Gomori, 1941) were used.

Immunocytochemistry

For this the immunoperoxidase method, i.e., the peroxidase-antiperoxidase (PAP) method of Sternberger (1979) were followed.

The PAP kit, K-512 (Glostrup, Denmark) was used for the unlabelled antibody enzyme method. The kit contained antibodies to human insulin, glucagon and somatostatin.

As positive control, human pancreatic tissue were processed and stained immunocytochemically using the same antisera.

Adipokinetic hormone (AKH) antisera

AKH antisera were a kind gift from Dr. H. Schooneveld (The Netherlands, Agricultural University). The antiserum code No. 241 was raised in rabbit against a synthetic analogue of AKH, the (Tyr) AKH (Schooneveld *et al.*, 1983) which is C-terminal specific. Code No. 433 AKH antiserum is N-terminal specific. They were used in dilutions of 1:500 and 1:1000 respectively in PBS. For this also PAP method of Sternberger (1979) was followed and stained in diaminobenzidine (DAB).

Negative control for insulin, glucagon, somatostatin and AKH

Negative controls included omission of all steps except staining to reveal residual endogenous peroxidases and also omission of first antibody to check unspecific binding of second antibodies and other compounds applied in the procedure.

RESULTS

Immunocytochemical localization of Neuropeptides

Glucagon

When immunoperoxidase method was used, glucagon-like immunoreactive cells were found in the fifth instar larval brain of *A. janata*. The immunopositive cells appeared in slightly rose red colour. They were found localised on either side of the pars intercerebralis region (Fig. 1). They are two in number, round in shape and 20–25 m diameter. These cells are not paraldehyde fuschin-positive.

The positive control slides showed glucagon secreting cells in rose red colour and the negative control slides showed no immunoreactivity.

Somatostatin

A group of somatostatin-positive immunoreactive cells were seen in the pars intercerebralis region of the brain (Fig. 2) when the PAP method was employed which were primarily localised at the protocerebral region of the brain. They are generally oval although some appeared elongated measuring 15–20 μm cell diameter. Some of the immunoreactive cells could also be localised outside pars intercerebralis (Fig. 3) region of the brain. These cells are smaller having 10μm diameter. These somatostatin positive cells at pars intercerebralis region corresponds to A-type NSCs stained by CHP (Fig. 4) and PF methods (Fig. 5) both in secretory content and in size. The CC-CA complex (Fig. 6) containing axonal endings of these cells also found to be stained by human somatostatin antiserum. The positive control sections also showed somatostatin secreting cells (Fig. 7) in rose red colour while the negative control ones remained blue due to the background haematoxylin counter staining.

Insulin

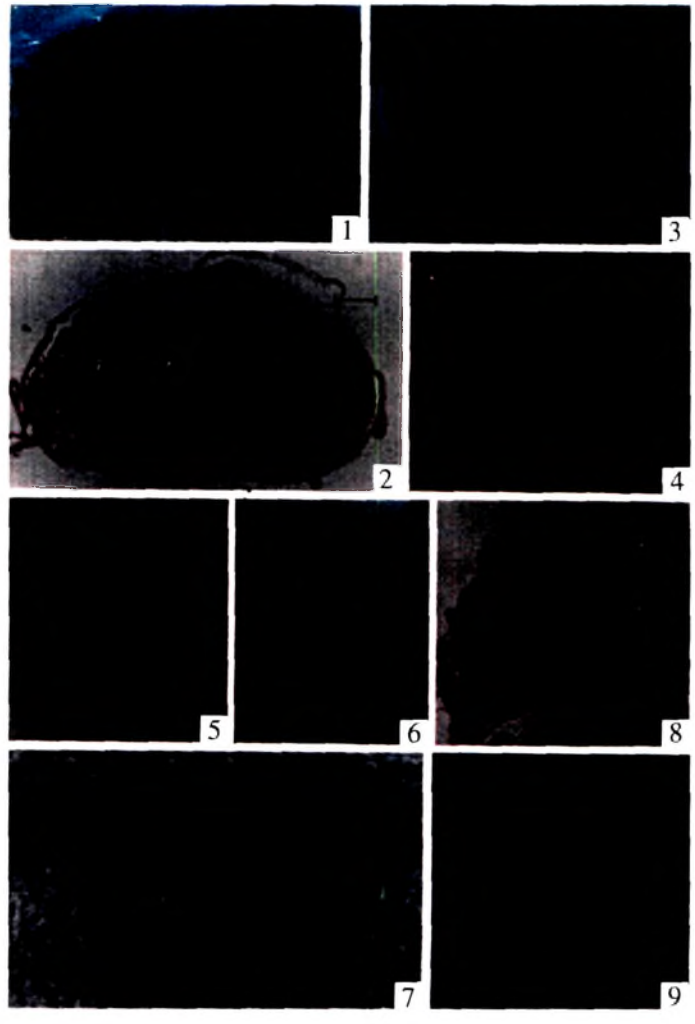
Sections stained for insulin like immunoreactivity showed negative reaction in the brain as shown in (Fig. 8).

Adipokinetic hormone

Although AKH-antisera No. 241 and 433 were used, positive staining was obtained only with 241-sera in the brain of *A. janata*. Three to four numbers of immunoreactive secretory neurons could be localised on the anterior dorsal side of brain using AKH-241 sera. The cells are somewhat rounded in shape and measured 20–25 μm in diameter (Fig. 9). The cells stained brown due to DAB stain. These neurons are not CHP or PF positive. The negative control slides showed negative results. AKH-433 like immunoreactivity was not seen in the brain-retrocerebral complex.

DISCUSSION

In the present study, fifth instar larvae of both sexes of *A. janata* were examined for glucagon-like immunoreactivity. Positive results were obtained when glucagon anti-



FIGURES. Histological section of brain *of A. janata* caterpillars showing: Fig. 1. Human glucagon-like immunoreactive cells near pars intercerebralis. 2. Human somatostatin-like immunoreactive neurons at the PIC region. 3. Somatostatin-like neurons outside PIC region. 4. Median neurosecretory cells stained with CHP. 5. Median neurosecretory cells stained with PF. 6. Retrocerebral complex stained with somatostatin antiserum. 7. Positive control slide of human pancreatic tissue showing agranular D-cells. 8. Section of brain of A. *janata* showing negative results with insulin anti-serum. 9. Neurons in the brain of A. *janata* stained with AKH-241 antiserum.

AKH - Adipokinetic hormone
CC-CA - Corpora Cardiaca-Corpora allata complex
G - Glucagon-like neuron
MNSC - Median neurosecretory cell
S - Somatostatin-like neuron
SSC - Somatostatin secreting cell
Bars 1–9 represent 40 m

serum was applied to sections of brain tissue of *Achaea* . The glucagon immunoreactive cells were located one on either side of the protocerebrum. The colour of immunoreactive neurons was not intense indicating a lesser amount of glucagon-like antigen in *Achaea* caterpillars. Since these were not PF-positive these cells were not corresponded to the A-type neurosecretory cells.

Similar results were obtained by El-Salhy *et al.* (1983) in *M. sexta* where he found two groups of glucagon-immunoreactive cells in the brain. The neurons were both pancreatic-glucagon and glicentin specic. Tager and Kramer (1980) demonstrated glucagon-like peptide in *M. sexta* and Duve and Thorpe (1984) localized glucagon-like peptide in the blowy *Calliphora vomitoria*. It was also seen in *E. aeneus* (El-Salhy *et al.*, 1980) and in the midgut of the cockroach *P. americana* (Iwanaga *et al.*, 1981).

The present nding of glucagon- positive immunoreactive neuron in *A. janata* indicate the presence of cells similar to human pancreatic glucagon producing cells. Glucagon-like peptides have now been detected by immunochemical or biological techniques in many species of invertebrates. Tissue sources included whole body, head, gut, hepatopancreas, brain, ganglia and haemolymph. There appear to be multiple forms of this peptide with ranging molecular masses depending on the tissue used for extraction.

Somatostatin is a growth hormone release-inhibiting peptide which suppresses the secretion of other hormones such as insulin, glucagon and gastrin affecting gastric and pancreatic exocrine secretions. In the present study, somatostatin-like immunoreactive cells could be localised both in the brain and in the retrocerebral complex of *A. janata* larvae. Those located near *pars intercerebralis* region correspond to A-type NSCs which were denitely different from glucagon-positive cells. The somatostatin-positive immunoreactive cells are more in number than glucagon-positive cells. In *A. janata*, another group of somatostatin-immunoreactive neurons were found out side PIC region which agrees with the results of El-Salhy *et al.* (1980) in *M. sexta*.

The present observation that somatostatin immunoreactive cells observed in *A. janata* brain emit nerve bres which are found to reach the neurohaemal organ CC suggests that these neuropeptides may be produced in the brain and transferred to the neurohaemal organ for storage and subsequently released into the circulating haemolymph as and when needed. This assumption gains some support from the fact that two of these GEP peptides viz. insulin and glucagon have been detected in the circulating haemolymph of insects (Kramer *et al.*, 1982) and that they display biological activities comparable to those of mammalian insulin and glucagon (Tager *et al.*, 1976). The possibility that some of these GEP neuropeptides might act as neurotransmitters and or neuromodulators and exert their effect on neighbouring cells in the brain or CC/CA cannot however be excluded.

All control reactions performed for the above demonstrations were negative implicating that the immunoreactions reported here were specic for the applied antisera and not cross reacting with each other.

In the present study no insulin-like immunoreactivity could be localised in the brain

of *A. janata* even if different fixation time for the insect tissue and varied incubation times with the antisera were tried. Anti-insulin not cross-reacting with antigens in the brain tissue of *A. janata* does not necessarily mean that this hormone is entirely absent in this insect. As antibodies to hormones are highly specific failure of cross reactivity with mammalian antisera could mean perhaps the lack of specific epitope in the antigen for the appropriate antiserum. The failure of any cross reactivity could also indicate structural differences between mammalian gut hormones and those produced in invertebrates. These results also points to the possibility that insulin like material in the brain of *A. janata* may not be identical to mammalian insulin.

Similar results were obtained by El Salhy *et al.* (1983) in adult *M. sexta* brain. He obtained immunoreactivity only when insulin B chain specific antiserum was used. The absence of insulin immunoreactivity in CC was reported in *Bombyx* (Yui and Oto, 1980), and in some other insects (Raabe, 1986) as well. Veenstra *et al.* (1985) used seven different insulin antisera which reacted specifically with pancreatic insular B-cells, and only one reacted more or less specifically with cells in the Colorado potato beetle.

Using AKH. 241 antiserum, immunoneurons could be localised in the brain of *A. janata*. Antiserum-433 could not reveal any immunoreactive neurons. It agrees with the results of Schooneveld *et al.* (1987b) in different species of insects where most of the neurons in the brain were left unstained with serum 433. In *Lymnaea, Porcellio, Lithobius* etc. also the neurons reacted with only AKH antiserum code 241 (Schooneveld *et al.*, 1987b). He suggested that serum 241 is specific for AKH where as serum 433 recognise AKH and AKH II. as well as their precursors. Like the present findings, two neurons in the brain were stained with AKH 241 in another Lepidopteran insect, *Galleria* (Schooneveld *et al.*, 1985) as well.

Method specificity was controlled by omission of each reagent, one at a time from the regular staining sequence in a normally performed PAP staining procedure, as well as by replacement of the primary antiserum by a normal rabbit serum. All of these treatment resulted in the complete absence of immunostaining.

Duve *et al.* (1983) opines that ICC studies with appropriate antibody specificity controls, the conclusion is that some insect neuropeptides have amino acid sequences in common with those of the antigenic determinants of the peptide hormones used to generate the antisera. Those parts of the insect peptides recognised by the antisera have been relatively stable during evolution, because these parts are essential for the functioning of these insect peptides. Studies so far indicate that neutopeptides can play a multitude of functional role in the brain and even single neuropeptides are likely to be multifunctional (Nassel and Homberg, 2006; Audsley and Weaver, 2009). It can be hoped that after identifying tissues containing regulatory chemicals, we can start the process of isolating and sequencing them and then study their mode of action and physiological roles.

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Effect of *Lantana camara* L. leaf extract on the larval development of *Sitophilus oryzae* L. and *Tribolium castaneum* H.

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ABSTRACT: *Sitophilus oryzae* L. and *Tribolium castaneum* H. are major pests of stored grains. Plant leaf extract of *Lantana camara* (Verbenaceae), was studied for their larvicidal effects on the stored grain pests *Sitophilus oryzae* L. and *Tribolium castaneum* H. Response varied with different doses. No mortality had occurred in the control. Significant larvicidal activity against *S.oryzae* and *Tribolium castaneum* H. larvae were achieved with ethanol extracts than the aqueous extracts. Also first instar larvae were more affected than the last instar larvae. Extracts from this naturally occurring plant could be useful grain protectants against *S.oryzae* and *T. castaneum*.
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KEYWORDS: *Sitophilus oryzae*, *Tribolium castaneum*, *Lantana camara*, plant extract, larvicidal effect, bio pesticides

INTRODUCTION

Safe storage of grains and food products against insect damage is a serious concern (Haq *et al*., 2005). It has been estimated that about 10% of the world's grain production is lost to post harvest insect and mite's infestations (Tooba *et al*., 2005) due to unfavourable climatic and storage conditions (Rahman *et al*., 2009). Though spectacular progress in pest control has been achieved by chemical measures, its adverse effect on ecological system and human life has stressed the need to develop alternative methods for controlling the various arthropod pests (Risk *et al*., 2001). The rice weevil is a serious pest of grain in the tropics and subtropics. It attacks cereal grains such as rice, wheat, corn, oats, maize, barley, sorghum, and also processed cereal products. *Tribolium castaneum* is commonly known as red flour beetle. It has six larval instars. It takes about 24–32 days to complete the life cycle at various

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temperatures. Their presence in stored foods directly affects both the quantity and quality of the food material (Mondal, 1994).

To control the pests in storages chemicals like methyl bromide (MeBr) and phosphine (PH3) are used, that may cause several problems (Negahban *et al.*, 2006). Therefore, alternative pests control methods, need to be developed. For example, a lot of natural pesticides can be isolated from higher plants. These products do not leave harmful residue to the environment and have medicinal properties for human, with lower toxicity to mammals (Negahban *et al.*, 2006).

Plants such as *Azadirachta indica* (A. Juss), *Cassia fistula* (L.), *Calotropis procera* (Ait), and *Chrysanthenum coronarium* (L.) have shown insecticidal, antifeedant, repellant and growth regulating properties (Deka and Singh, 2005; Singh and Singh, 2005; Prakash and Rao, 2006; Kestenholz *et al.*, 2007; Neoliya *et al.*, 2007; Sankari and Narayanaswami, 2007). They are traditionally and widely used as stored grain protectants due to their easy accessibility and biodegradable nature (Dwivedi and Garg, 2003).

Plants constitute an important source of active natural products which differ widely in terms of structures, biological properties and mechanisms of actions. In recent years, the extracts of many plants have been screened for their anti insect activities. The presence of secondary compounds, which of known function in photosynthesis, growth or other aspects of plant physiology, give plant materials or their extracts their anti-insect activity.

Secondary compounds include alkaloids, terpenoides, phenolics, flavonoids, chromenes and other minor chemicals. They can affect insects in several different ways and cause rapid death, act as attractants, deterrents, phagostimulants, antifeedants or modify oviposition. They may retard or accelerate development or interfere with life cycle of insect in other ways. Hence, the industrial utilization of medicinal and aromatic plants should be undertaken with conservation measures in mind.

The plant selected here is *Lantana camara* L. (Family: Verbenaceae). It is a significant weed of 650 varieties in over 60 countries. It is a low erect shrub with prickles and a strong odour. *L.camara* has several uses mainly as a herbal medicine and in some areas as firewood and mulch (Dey *et al.*, 2003). Plant extracts are used in folk medicine for the treatment of cancers, chicken pox, measles, asthma, ulcers, swellings, tetanus, rheumatism, malaria, etc. (Begun *et al.*, 1995). Because of these evidences we have selected this plant to be used as an insecticide against stored product pests.

MATERIALS AND METHODS

1. Test insects

Sitophilus oryzae L.

The rice weevil is a small dark brown weevil with a long snout, and ridges along the wing covers. The larvae are white or creamy white grubs with a small light brown head. Length of the larvae varies from 2.5 mm to 3. They are often found in grain



FIGURES 1. *Lantana camara* L.

storage plants and processing plants. The female weevil bores a tiny hole in the grain kernel and deposits an egg inside. The larva develops and pupates within the grain kernels and is rarely seen. The adult weevil emerges from the pupa and cuts an exit hole to emerge. The exit holes of the rice weevil are smaller than those of the Granary weevil, and are smoother edged. Sometimes the adult lays eggs in the surface of the grains and the larvae can be seen outside the kernels. The rice weevil is originally from Asia, but is now found around the world as a pest species transported in grain.

Tribolium castaneum H.

Red our beetles have chewing mouthparts, but do not bite or sting. The beetles are $\frac{1}{4}$ inch long; the adults are long-lived and may live for more than three years. The eggs are white, microscopic and often have bits of our stuck to their surface. The slender larvae are creamy yellow to light brown in colour. The pupae are lighter in colour, being white to yellowish. These beetles can breed throughout the year. The beetles give off an unpleasant smell, and their presence encourages mould growth in our.

Culturing of test insects

Initial stock was obtained from infested grains and our bought from the local market. The adult insects were introduced into the raw rice and wheat our. The culture was maintained in the laboratory. The cultures were cleaned everyday to avoid contamination. First and last instar larvae were collected for the experiments.

Source and preparation of plant materials

The lantana camara selected here is the wild variety with orange owers (Figs. 1). Leaves of the plants were collected from the surrounding areas. The leaves collected

were cleaned, chopped and air dried under room temperature (27 C) for seven days. When fully dried, they were separately ground into powder and kept in air tight jars prior to use. Plant extracts were prepared from the powdered leaves using the soxhlet apparatus. Ethanol and distilled water was used as the solvent for ethanol and aqueous extracts respectively. Duration of the extraction was extended up to 6–8 hours.

Treatments

Residual Film method was used for the studies. No. 1 what man lter paper cut in to round shape was deposited in the bottles (containers with lid-diameter 5 cm, height 2 5 cm). The leaf extract diluted to different concentrations (1%, 2%, 3%, 4% and 5% respectively) were applied to the lter paper and allowed to dry. 20 gm fresh feed (raw rice thoroughly washed and dried or wheat our) was taken in the container. 10 larvae were deposited in the container. Control was prepared in the same way. The extract application to the lter paper was omitted in the control. Instead of extracts, solvents were added. Five replicates were set up for the control and the treated. The development of the larvae were assessed and recorded every day, up to adulthood.

Statistical analysis

Data were subjected to analysis of variance (one way ANOVA) test using SPSS (windows 10), statistical software.

2. Observation and Results

Effects on larval weight in *S. oryzae*

The mean SE *n* 10 weight of last instar larvae was 2 0.1 which increased gradually and reached nearly 20 mg (2 mg per each adult) at the time of adult emergence. In the case of rst instar larvae the mean SE *n* 10 weight was 0.5 0.01. Control larvae had shown an increase in individual weight during 5–7 days. The weight of the treated larvae showed reduction in 7–8 days. It is observed that the larvae which lost their weight in 7–8 days lost more weight and were found dead within 10–14 days. The mean SE *n* 10 weight of last instar larvae was 1 0.1 and that of rst instar larvae was 0.2 0.1 in the experimental culture.

Effects on larval weight in *T. castaneum*

The mean SE *n* 10 weight of last instar larvae was 2.7 0.1 which increased gradually and reached nearly 20 mg (2 mg per each adult) at the time of adult emergence. In the case of rst instar larvae the mean SE *n* 10 weight was 1.5 0.01. Control larvae had shown an increase in individual weight during 5–7 days. The weight of the treated larvae showed a reduction in 7–8 days. It is observed that the larvae which lost their weight in 7–8 days lost more weight and were found dead within 10–14 days. The mean SE *n* 10 weight of last instar larvae was 1.5 0.1 and that of rst instar larvae was 0.8 0.1.



FIGURES 2. Dead larvae of *T. castaneum* when treated with *L.camara* leaf extract

Effects on larval mortality

It was observed that there was no mortality in control set up. But all the treated set up had shown a signicant mortality rate. Table 1 explains the mortality rate in treated larvae. Mortality rate was higher in the rst instar larvae than in the last instar larvae. Also the mortality rate was higher when treated with ethanol extracts. The mortality rate was higher in the *T. castaneum* when compared to the *S. oryzae*. The dead larvae had shown loss of weight in both pests. Colour of the larvae was changed signicantly in *T. castaneum* (Fig. 2).

Effects on the emergence rate

The effect of extracts on the emergence rate of *Sitophilus oryzae* and *T. castaneum* had shown that it reaches 100% in the control. Whereas the emergence rate in the treated diet was signicantly low. The larvae which pupated without losing their weights emerged as adults (Fig. 3 & 4).

Effects on the larval period

In control larval duration was 7–15 days and there was no signicant difference in the developmental period of the treated larvae. The pupated larvae in the treated diet also took the same time to emerge as adults in both pests.

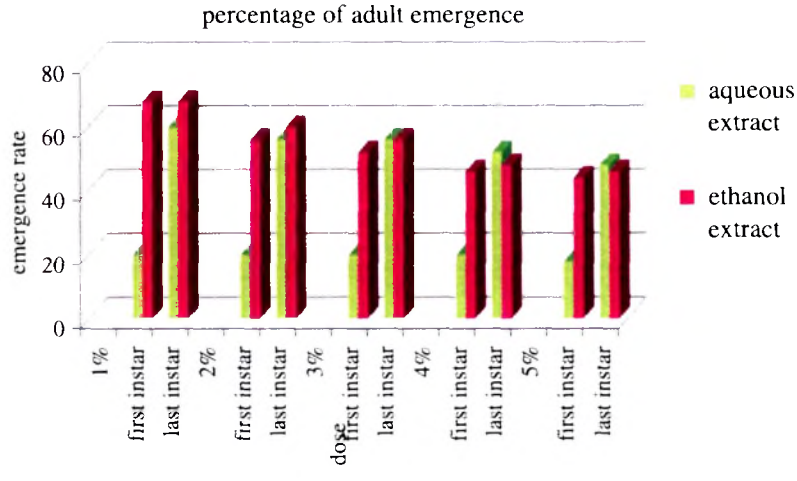
DISCUSSION

The results revealed that *L. camara* leaf extract has larvicidal properties, which can be used as an environment friendly biopesticide. Signicant larvici dal activity against *T. castaneum* and *S. oryzae* larvae were observed with powdered leaves of *L. camara*. (Susha and Dhaniya, 2010). The results were similar in both the pests *T. castaneum*

TABLE 1. Mortality of rst instar and last instar larvae of *Sitophilus oryzae* and *Tribolium castaneum* when treated with plant extract.

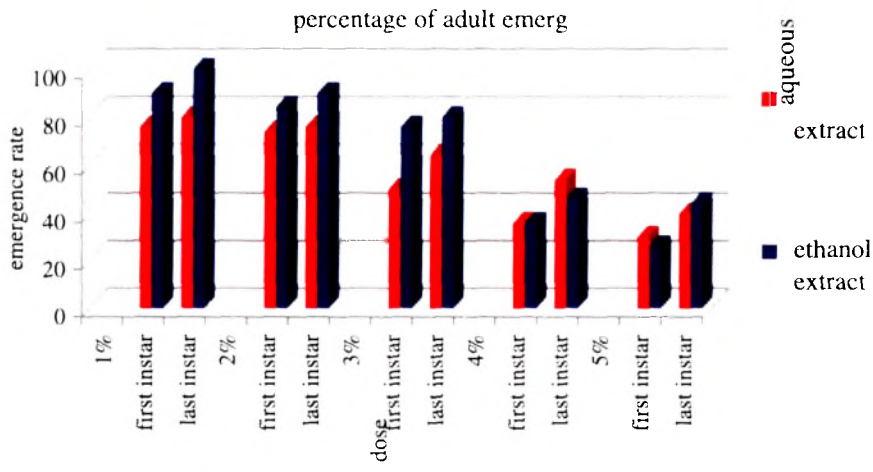
Dose	Age of insects	*Mortality rate*				*Mortality rate*			
		S.oryzae		*T. castaneum*		*S.oryzae*		*T. castaneum*	
1%	First instar	1.94	0.09	1.47	0.06	1.82	0.13	1.37	0.16
	Last instar	1.71	0.13	1.71	0.13	1.72	0.09	1.00	0.00
2%	First instar	2.08	0.12^{a}	1.60	0.07^{b}	1.86	0.17^{a}	1.56	0.20^{a}
	Last instar	1.78	0.05^{a}	1.93	0.12^{b}	1.88	0.10^{a}	1.37	0.16^{a}
3%	First instar	2.44	0.06^{bb}	1.72	0.09^{ab}	2.44	0.06^{bb}	1.82	0.13^{bb}
	Last instar	2.04	0.09^{bb}	2.13	0.12^{ab}	2.13	0.12^{bb}	1.96	0.18^{bb}
4%	First instar	2.79	0.06^{bbb}	2.57	0.04^{bbb}	2.70	0.13^{abb}	2.70	0.04^{bbb}
	Last instar	2.36	0.05^{bbb}	2.36	0.05^{bbb}	2.36	0.08^{abb}	2.52	0.08^{bbb}
5%	First instar	2.84	0.17^{abbb}	2.72	0.04^{abbb}	2.67	0.10^{aabb}	2.79	0.07^{abbb}
	Last instar	2.44	0.06^{abbb}	2.53	0.04^{abbb}	2.44	0.11^{aabb}	2.48	0.07^{abbb}

* Each datum represents the mean S.E of ve replicates. n 10 .
Means within a column followed by the different letters are highly signicant p 0 05 .



FIGURES 3. Percentage of adult emergence in *S. oryzae*

and *S. oryzae*. But the mortality rate was higher in *T. castaneum*. This may be due to the occurrence of larvae in the our. *S. oryzae* larvae are frequently found inside the kernels. The rst instar larvae were more susceptible to plant extracts than the last instar larvae. These results agree with the previous results obtained during the preliminary studies. Results of this study thus demonstrated that effect of the plant extract decreased with the increase of the age of the larvae.



FIGURES 4. Percentage of adult emergence in *T. castaneum*

In the present study all the larvae pupated had emerged into adults. It was observed that larvae that did not pupate had shown decrease in the bodyweight. It was also observed that that plant powders have not induced any mortality in the pupae. This is in agreement with earlier studies. Bell (1978) showed that pupae may exhibit a higher tolerance to chemical agents than active stages. However the leaf extract had no effect on the duration of the larval period. Control and treated larvae took almost the same time to emerge as adults. In control larval duration was 15 days. It was the same duration in all the live larvae recovered from cultures containing extract treated samples.

The emergence of adult insects from all control samples indicated that there may be different compounds in extracts possessing different bioactivities. From the emergence adult insects from the treated samples it is evident that tested insects were capable of effective oviposition and that of prevention of adult emergence is due to treatment.

Various reports are available that the secondary metabolites of the plants can affect insects in several different ways. They may act as attractants, deterrents, phagostimulants, antifeedants or they might modify oviposition. They may retard, accelerate development or interfere with the life cycle of the insect in other ways (Wink *et al.*, 2004).

Also treatments using the ethanol extracts had shown more mortality rate than the aqueous extracts. EI-Sebae *et al.* (2008) reported that this may be due to variations in the type of active ingredients and its chemical structure and their mode of toxic action exerted by their aqueous or organic extracts. This result is also in agreement with El-Doksh *et al.* (1984) who reported that the LD_{50} values of the organic extract were more toxic than LD_{50} values of the aqueous extract, and that was due to the increasing of effective components in organic extracts in most plants.

Chemical constituents of different *camara* species had been extensively studied by many people. All these studies have revealed the presence of terpenoids, steroids, and alkaloids as major chemical constituents in Lantana camara (Saleh, 1974; Siddiqui *et al.*, 1995; Deepak *et al.*, 2009). Barre *et al.* (1997) reports that the lantadenes present in all the lantana species may be responsible for the biological activities. In addition, other secondary metabolites such as alkaloids, terpenoids and phenols could be partially responsible for some activities.

CONCLUSION

L.camara extract had shown high mortality rate in *Sitophilus oryzae* and *Tribolium castaneum*. The plant being medicinal provides a good chance for using against these stored product pests. Since it is available everywhere, it can be used by all people without any nancial support. It can be used as an environment friendly insecticide. The concentration of plant extract used is very low in these studies. The higher concentration may be more effective. Further studies including eld trials are going on.

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Influence of formic acid on the biology of *Oecophylla smaragdina* (Fabricius)

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ABSTRACT: Apterous workers and winged reproductive forms of *Oecophylla smaragdina* possessed formic acid (FA) in their body. Among the worker castes, minor workers which are fully confined within the nest had highest amount of FA. The content of FA in major worker was 9.7 ± 0.7 mg/gm tissue which was 1/4 of the content of minor workers, 1/3 of the content of intermediate workers and 10 times higher than that of non biting reproductive forms. Developing forms such as larvae and pupae had no traces of FA in the body. FA was present in the poison gland reservoir within the abdomen of workers and it was transferred in between the mandibles and kept as a tiny drop at the time of aggression. During the 24 hour cycle, the major workers followed a biochemical rhythm in the content of FA which was low in the morning and night with a peak at noon which gradually decreased towards evening. Major workers, when allowed to bite continuously for more than 15 minutes at a particular site on the body resulted in death of the ant at which time the FA content was decreased to non detectable amounts and body weight was reduced to 60% of the original weight. Continuous disturbance caused an increase in the content of FA in worker ants.

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KEYWORDS: *Oecophylla smaragdina*, formic acid, aggressive behaviour, death of biting ants, biochemical rhythm of FA

INTRODUCTION

The Asian arboreal weaver ant *Oecophylla smaragdina* is one of the most common and of the most dominant ant colonies which form a very elaborate communicative organization in the insect world which are known for their painful irritating bite. They are generally active and fast moving and will defend their nest and territory vigorously

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by attacking the intruders with the help of mandibles and formic acid (FA) Holldobler, 1983; (Holldobler and Wilson, 1977). Typically a colony possess winged males, winged females, developing stages and morphologically different sizes of workers (Rossy, 1986). A mature colony of weaver ants can hold as many as 500000 workers and may span as many as 150 nests (Holldobler and Wilson, 1990). The predatory power of *O. smaragdina* is most outstanding among ants in their localities (Way and Khoo, 1992; Peng *et al.*, 1997, 1999; Mele and Cuc, 2000). They can protect the host plants from attacks of phytophagous insects and therefore had been used as pest control agents. The formicine ants had very high content of FA and reports suggested that FA is formed from the amino acid pool of glycine, serine and histidine (Hefetz and Blum, 1978). Even though there are reports that FA is present in high concentration in various formicine ants (Lopez *et al.*, 1993), there is no detailed study on its presence in different colony individuals such as winged males, winged females, sterile workers and developing stages and also its influence on the biology of *O. smaragdina*.

MATERIALS AND METHODS

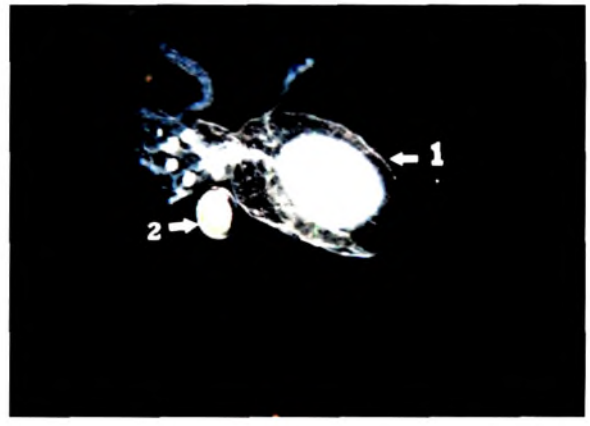
The ants were collected from the wild with minimum disturbance by putting a medium sized nest in a plastic container which was applied with cotton soaked in chloroform. The mouth of the jar was closed immediately with the lid and after 5 minutes the colony individuals were dropped on a white paper. The different individuals of the colony were separated and weighed. The FA content of the different colony individuals were estimated as described by Colowick and Kaplan (1963). The body of the worker ants were cut using a sharp blade, and separated as head, thorax and abdomen. After that the FA content was estimated using each of these body parts. The FA content of the workers at different time intervals was determined by assaying FA content present in the morning, afternoon, evening and at night.

Abdomen of major workers were dipped in ice cold distilled water for 2 minutes, after that fine surgical needle was inserted in to the tip of abdomen, the glands were exposed by gentle pull and viewed under dissection microscope. Photos of poison gland and Dufour's gland were taken using Sony Cyber-shot 4x optical zoom camera

In order to know the importance of FA in the behavior of ants, they were disturbed for 15 minutes and after that it was estimated. The major workers were allowed to bite continuously at a particular body site for more than 30 minutes and after that the FA content was estimated. Statistical analysis were done for assessing the significance of results (Daniel, 2006).

OBSERVATION AND RESULTS

The observation on an exposed nest revealed that the polymorphic colony of *O. smaragdina* possessed winged males, winged females and three types of apterous workers (major, intermediate and minor). FA was synthesized by the poison gland and was stored in the reservoir until it was charged on the victim. In association with poison gland, an another small gland called Dufour's gland is located (Fig. 1) in the



FIGURES 1. Poison gland, 2-Dufour's gland. Dissected glands viewed under dissection microscope (20x) with camera Sony Cyber-shot 4x optical zoom.

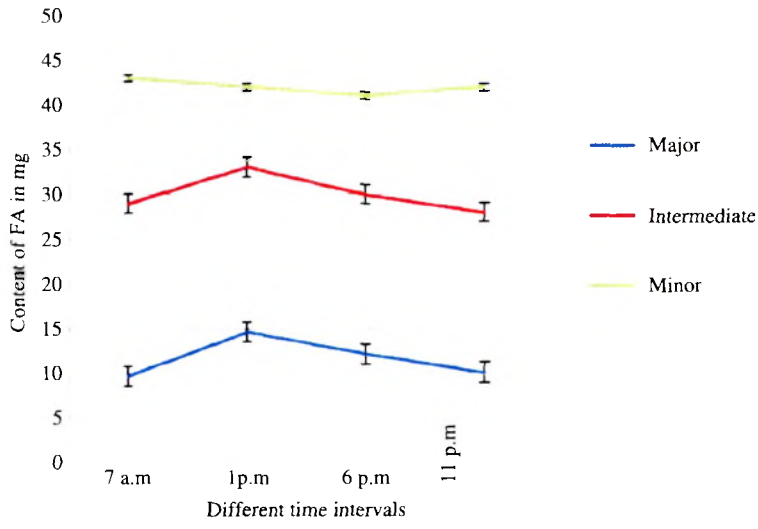
TABLE 1. The content of formic acid in different individuals in the colony of *O. smaragdina*.

Individuals		Formic acid	
Workers	Major	9.7	0.7*
	Intermediate	29.1	2.3*
	Minor	43.7	3.1*
Major Worker	Head	0.63	0.02
	Thorax	0.11	0.01
	Abdomen	42.9	3.6
Winged Forms	Males	1.27	0.12
	Females	1.23	0.11
Developing Forms	Larva	Nil	
	Pupa	Nil	

All values are mean SD, *n* 6.
Values are expressed in mg/gm fresh tissue.

abdominal cavity. Among different individuals of the colony worker castes possessed high content of FA but the winged males and females possessed very low content of FA. Among the worker castes, minor workers possessed very high content of FA followed by intermediate and major workers. The head and thorax of major workers possessed negligibly low content of FA but developing stages such as larvae and pupae had no traces of FA (Table 1).

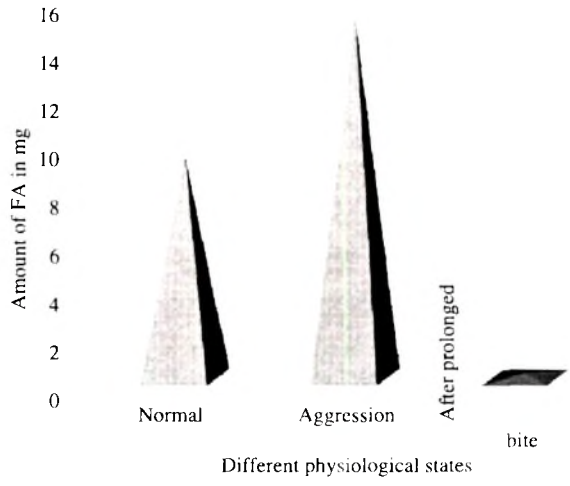
FA has a strong inuence on the biology of these ants. On estimating FA of worker castes at different time intervals, it was found that the FA of major workers showed a strong periodicity with physical activity (Fig. 2). In major workers FA during afternoon (1 p.m) showed a 50% elevation when compared with the contents



FIGURES 2. Fluctuations of FA content of different worker castes of *O. smaragdina* in 24 hour cycle. All values are mean SD, *n* 4. Values are expressed in mg/gm fresh tissue. Elevation of FA content in major and intermediates at 1 p.m were signicantly different from FA content at morning. *p* 0 01, *n* 6.

at morning (7 a.m). The value of FA gradually decreased at evening and reached at its original value at night. The intermediate workers also showed a slight but signicant elevation of FA at 1 p.m but its uctuation were not prominent as in major workers The minor workers did not show any signicant uctuation in the content of FA during different times of 24 hour cycle (Fig. 2).

When the major workers were allowed to bite, most of them detached from the site of bite and searched new sites. After three or four bites they did not show any interest in biting a new site. A small number of ants did not detached from the site of bite and continued on biting for more than 30 minutes. These ants when forcefully detached from bite site, they showed the lack of co-ordination in movements and nally died. The body weight of a major worker with body weight of 11.8 0.8 after 30 minutes of prolonged biting was reduced to 60% of its original weight and it was 6.6 0.4 mg. The major workers after 30 minutes of prolonged biting did not possessed any traces of FA in their body (Fig. 3). Disturbance made on the nest, resulted that a sharp increase in the content of FA in major workers. It was observed that most of the major and intermediate worker castes came out of the nest with a tiny drop of FA in between the mandibles with threatening position by rising front pair of legs. During the course of bite the worker ants intermittently constricted their mandibles with a simultaneous spray of FA by forward bend of abdomen at the bite site (Fig. 4).



FIGURES 3. Variation in the content of FA in relation to different physiological states of major workers of *O. smaragdina.* Values are expressed as mg/gm fresh tissue. All values are mean SD, *n* 6.



FIGURES 4. Biting Posture of *Oecophylla smaragdina.*

DISCUSSION

Even though the previous investigators (Holldobler, 1983; Holldobler and Wilson, 1977, 1990) have reported only two types of workers in the colony of *O. smaragdina* (major and minor), we are suspecting the presence of a third type of worker, an intermediate one (Vidhu and Evans, 2010) with clear difference in the content of FA, which are smaller than major workers and larger than minor workers (Table 1).

FA is present in the poison gland seen in association with Dufour's gland in the abdomen of workers (Fig. 1). Detection of low quantities of FA in the head and thorax

of ants was actually due to contamination and the fact was ruled out through detection of no traces of FA in head and thorax after repeated washings in distilled water. Among the workers the minor workers possessed very high content of FA when compared with other two types of workers. The winged males and females exhibited some amount of FA which was higher than that of the amount present in head and thorax of major workers (Table 1). The adult males and females did not show any tendency to bite while handling. It was reported that the winged males and females at the time of rain make a nuptial ight and after that the fem ale shed their wings and start a new colony (Sudd, 1967). Traces of FA present in the body of reproductive females may help them from predation during their reproductive phase as a solitary, apterous organism.

The FA present in the workers had a profound inuence on the activity of rhythm of these ants. Circadian rhythms in the activities of four societies of *O. longinoda* have been studied. It was shown that the activity is continuous, but diurnal activity is greater than the nocturnal activity (Dejean, 1990). Being it a poikilothermic organism the physical activity is related with temperature. It was reported that in *O. smaragdina* temperature has profound inuence on the foraging activity (Rossy and Narendran, 1988). Hence it can be concluded that the FA content in the body of major workers is directly proportional to the physical activity such as predation, maintenance of territory and scavenging (Fig. 2). It was observed that these ants became very active in sunny days especially after one or two days of rain, at which time the temperature and humidity will be high. The minor workers were mostly conned with in the nest itself and thus not encountering with preys and intruders and hence FA content didn't show any uctuation during the 24 hour cycle. The role of extremely high content of FA in small, weak and delicate organisms which are almost fully conned within the nest is very interesting. Minor workers have an important role in nursing the brood (Holldobler and Wilson, 1990) and hence the FA present in them may have role in protection of brood from microbes. It is reported that FA has antimicrobial activity and it is used as an antibacterial agent in poultry industry and in live stock management (Thompson and Hinton, 1997).

It was reported that in worker ants of formicine ant *Formica rufa*, the formic acid and some hydrocarbons have a combined effect and their relative concentrations regulate the intensity and duration of the alarm behavior. In case of *O. smaragdina* the content of the FA in the body of workers were very much related to their behavior (Lofqvist, 2003). When there was continuous aggression which resulted sharp elevation in content of FA with transfer of it from abdominal end to mandibles together with increased production of FA (Fig. 3). Worker ants on prolonged biting showed absolute loss of FA and at the same time they died in due course. Even though FA is a secondary metabolite produced as a defense material, it's very presence is essential for the survival of ant itself.

ACKNOWLEDGEMENT

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New combination and synonyms of aphids (Hemiptera: Aphididae) described from India

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ABSTRACT: *Ceratovacuna indica* Ghosh, Pal and Raychaudhuri is treated here as *Astegopteryx indica* (Ghosh, Pal and Raychaudhuri), comb. nov. *Eutrichosiphum assamense* Ghosh, Basu and Raychaudhuri and *Eutrichosiphum simlaensis* Ghosh are considered as new junior synonyms (syn. nov.) of *Eutrichosiphum khasyanum* (Ghosh and Raychaudhuri). © 2011 Association for Advancement of Entomology

KEYWORDS: Aphid, Hemiptera, new combination and synonyms, India

INTRODUCTION

One species described in the aphid genus *Ceratovacuna* Zehntner (Subfamily Hormaphidinae) and 5 species and 1 subspecies in *Eutrichosiphum* Essig and Kuwana (Subfamily Greenideinae) were studied and the validity of these species have been ascertained in this paper.

Abbreviations: – apterae: apterous viviparous female; alatae: alate viviparous female; u.r.s: Ultimate rostral segment; h.t.2: second segment of hind tarsus; p.t: processus terminalis.

Astegopteryx indica (Ghosh, M. R., Pal & Raychaudhuri) comb. nov.

Ceratovacuna indica Ghosh, M. R. *et al.*, 1977. *Proceedings of the Zoological Society*, Calcutta, 27: 96; Raychaudhuri *et al.*, 1980. *Zoological Society of Calcutta*: 370; Ghosh, A.K., 1988. *Zoological Survey of India*: 312.

Astegopteryx indica (Ghosh, M. R., Pal & Raychaudhuri) **comb. nov.**

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Specimens examined:

8 apterae (Holotype and paratypes), Mangpu, W.B., 9.ii.1971, on unidentified species of Bamboo, M. R. Ghosh coll.

Distribution:

India: West Bengal.

Remarks:

Ghosh, M. R., Pal & Raychaudhuri (1997) while describing *Ceratovacuna indica* from eastern India indicated the presence of many fine hairs on siphuncular cone of this species and compared this species with those under *Astegopteryx* Karsch, particularly with regard to the shape of wax gland cells and their arrangement on different parts of body, besides its cone shaped siphunculi. In addition, wax glands of this species are arranged in a longitudinal, frequently pressed close to each other and rectangular with rounded corners. However, in *Ceratovacuna* siphunculi are without hairs and wax glands transversely fused and wax cells present in two tires on 8th tergite. Ghosh, A. K. (1988) provided a description along with a dorsal view of apterae showing arrangement of wax glands on the abdomen. It shows that *C. indica* does not belong to *Ceratovacuna* Zehtner and should be included under *Astegopteryx* Karsch. Examination of type material confirms our observation. Accordingly, *Ceratovacuna indica* Ghosh, Pal and Raychaudhuri is considered as *Astegopteryx indica* (Ghosh, Pal and Raychaudhuri), new combination.

***Eutrichosiphum khasyanum* (Ghosh and Raychaudhuri)**

Eutrichosiphum (*Neoparatrichosiphum*) *khasyanum*: Raychaudhuri *et al.*, 1980. *Zoological Society of Calcutta*: 334.

Paratrichosiphum (*Neoparatrichosiphum*) *khasyanum* Ghosh, A. K. and Raychaudhuri, 1962. *Journal of Asiatic Society*, Calcutta, 4(3&4): 108; Saha and Chakrabarti, 1986. *Records of Zoological Survey of India*, 83(3&4): 3; Chakrabarti, Ghosh and Raychaudhuri, 1972. *Oriental Insects*, 6(3): 395.

Paratrichosiphum tattakanum assamensis Ghosh, A. K. and Raychaudhuri, 1962. *Journal of Asiatic Society*, 4(3&4): 112; Raychaudhuri *et al.*, 1980. *Zoological Society of Calcutta*: 335; Chakrabarti and Maity, 1980. *Annales Zoologici*, 35(9): 100; Maity, Bhattacharya and Chakrabarti, 1980. *Science and Culture*, 46: 312; Mandal, Saha and Chakrabarti, 1986. *Records of Zoological Survey of India*, 83(3&4): 3. syn.

Eutrichosiphum assamense Ghosh, Basu and Raychaudhuri, 1969. *Oriental Insects* 3(3): 247: Raychaudhuri *et al.*, 1980. *Zoological Society of Calcutta*: 330; Chakrabarti and Maity, 1980. *Annales Zoologici*, 35(9): 100; Maity, Bhattacharya and Chakrabarti, 1980. *Science and Culture*, 46: 312; Mandal, Saha and Chakrabarti, 1986. *Records of Zoological Survey of India*, 83(3&4): 3; Ghosh and Agarwala, 1993. *Zoological Survey of India*: 84. **syn. nov.**

Eutrichosiphum taoi Ghosh, Basu and Raychaudhuri, 1970. *Oriental Insects*, 4: 68; Saha and Chakrabarti, 1986. *Records of Zoological Survey of India*, 83(3&4): 3. **syn.**

Eutrichosiphum garhwalense Maity and Chakrabarti, 1980. *Annales Zoologici*, 35: 307; Saha and Chakrabarti, 1986. *Records of Zoological Survey of India*, 83(3&4): 3. **syn. nov.**

Eutrichosiphum simlaensis Ghosh, L.K. 1986. *Zoological Survey of India*, Calcutta. Technical Monograph, 16: 159. **syn. nov.**

Specimens examined:

5 apterae, 4 alatae, Shillong, 12.vii.1966, ex *Quercus fenestrata*, S. Biswas coll. (identified as *E. assamense*); 8 apterae, 4 alatae, 14 nymphs, Gourikund, 31.v.1978, ex *Quercus* sp., D. K. Bhattacharya coll. (identified as *E. assamensis*), 15 apterae, 26 alatae, 8 alate oviparae, 50 alate males and 31 nymphs, Mussoorie, Uttarakhand, 16.x.1976, ex *Quercus* sp., S. P. Maity coll. (identified as *E. assamensis*); 5 apterae and 14 nymphs, Trijugimarayan, 5.vi.1978, ex *Quercus* sp., S. Chakrabarti coll.; 16 apterae, 4 alatae, Mussoorie, 24.v.1978, ex *Quercus* sp., S. P. Maity coll.; 11 apterae and nymphs, Gourikund, 31.v.1978, from *Quercus* sp., D. K. Bhattacharya coll. (Holotype and Paratypes of *E. garhwalense*); 2 apterae, Khasya & Jayantia Hills, Assam, 10.vi.1961, ex *Quercus* sp., without collectors name (Holotype and Paratype of *E. khasyanum*); 6 apterae and nymphs, Bhowali, Uttar Pradesh, 5.iv.1970, ex *Quercus* sp., S. Chakrabarti coll. (identified as *E. taoi*).

Disribution:

India: Arunachal Pradesh, Assam, Himachal Pradesh, Meghalaya and Uttarakhand.

Remarks:

Ghosh and Raychaudhuri (1962) described *Paratrichosiphum khasyanum* sp. nov. from Khasya and Jayantia Hills on *Quercus* sp. under a newly erected subgenus *Neoparatrichosiphum* of the genus *Paratrichosiphum* Takahashi. In the same paper they also described a new subspecies *Paratrichosiphum tattakanum assamensis* from Shillong, Meghalaya. Ghosh, Basu and Raychaudhuri (1969) described another new species, *Eutrichosiphum assamense* from Shillong, Meghalaya. Raychaudhuri and Chatterjee (1980) considered *Paratrichosiphum* and *Neoparatrichosiphum* as subgenera of *Eutrichosiphum* and considered *Paratrichosiphum tattakanum assamensis* (misspelled as *assamense*) as a synonym of *Paratrichosiphum khasyanum* which was placed under *Eutrichosiphum* (*Neoparatrichosiphum*). *Eutrichosiphum assamense* Ghosh, Basu and Raychaudhuri remained as a distinct species under *Eutrichosiphum*.

Ghosh and Agarwala (1993) preferred not to separate the subgenera of *Eutrichosiphum* since the number of antennal segments and apices and length of siphuncular hairs are variable even in a single collection of many species of *Eutrichosiphum*. They (op.cit) also considered *E. garhwalense* Maity and Chakrabarti described from Uttar Pradesh (now Uttarakhand) and *E. taoi* Ghosh, Basu and Raychaudhuri described

TABLE 1. Comparison of some species of *Eutrichosiphum* described from India.

Characters	Species of *Eutrichosiphum*				
	assamense	*garhwalense*	*khasyanum*	*simlaensis**	*taoi*
Ant /Body	0.50	0.53–0.62	0.55	0.47–0.5	0.50
p.t /Base	1.33	1.330–1.6	1.1	1.3–1.6	1.0
Hairs on antennal segment III/IV/V	8/4/4	10/2/4	10/2/4		10/4/4
U.r.s/h.t.2	3.25–3.33	2.25–2.53	2.7–3.0	2.2–2.5	2.0–2.5
Hairs on head	4/3/2	4/3/2	4/3/2		4/3/2
Secondary hairs on u.r.s	6–8	12	10	6	10
Abdominal texture	Spinulose	Spinulose	Sclerotic, spinulose	Sclerotic, spinulose	Sclerotic, faintly spinulose
Hairs on 8th abdominal segment	2	2	2	2	2
Siphunculi /Body	0.2–1.25	0.28–0.33	0.25–0.27	0.27–0.29	0.25
Siphunculi /its max. width	3.1–3.4	3.6–4.0	5.1–5.5	3.5	4.1
Siphunculi colour	Pale yellow	Pale brown	Pale brown	Pale brown	Pale
Caudal hairs	7–8	4–6	9	6	7–8
Genital plate hairs	9	8	8		8
Femora	Spinulose	Spinulose	Spinulose	Spinulose	Smooth
Tibiae	Smooth	Smooth	Smooth, without apical tuft spines	Smooth with spiny hairs	Smooth
Length of siphunculi/width of head	-	1.14–1.20	1.15–1.20	-	-
Width of siphunculi/width of tibiae	2.00–2.10	1.75–2.00	1.50–1.80	1.40–1.60	1.7–1.9

*Taken from original description since types were not available from the author as well as from the Zoological Survey of India.

from Khasya and Jayantia Hills, Assam (now Meghalaya) and latter collecetd from different localities of Eastern and Northwest Himalaya respectively as synonyms of *Eutrichosiphum assamense* Ghosh, Basu and Raychaudhuri. Blackman and Eastop (1994) opined that *Eutrichosiphum garhwalense* Maity and Chakrabarti (1980) described from Uttar Pradesh, India on *Quercus incana* and *Eutrichosiphum simlaensis* Ghosh (1980) described from Simla, Himachal Pradesh may be synonyms of *E. khasyanum*. Remaudiere and Remaudiere (1997) also questioned the validity of *E. assamense* Ghosh, Basu and Raychaudhuri, *E. garwhalense* Maity and Chakrabarti, *E. simlaensis* Ghosh and *E. taoi* Ghosh, Basu and Raychaudhuri.

As because of this ambiguity regarding the validity of these species we have further studied the representative available specimens of all these species identified by different workers from different localities and also the available type specimens and a comparative data has been presented in the Table 1.

From the present comparative study, we not only agree with Ghosh and Agarwala (1993) regarding the synonyms of *E. garhwalense* and *E. taoi* with *E. khasyanum* but also confirm the speculation of Blackman and Eastop (1994), and Remaudiere and Remaudiere (1997) and propose *E. simlaensis* as a new junior synonym of *E. khasyanum*. Besides, we also propose that *E. assamense* is also a new synonym of *E. khasyanum*. Thus *E. assamense*, *E. garhwalense*, *E. simlaensis* and *E. taoi* become junior synonyms of *E. khasyanum*.

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Comparative study on antioxidant defence system in the bivoltine and trivoltine pupae of tasar silkworm, *Antheraea mylitta* Drury

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ABSTRACT: Daba ecorace of the tropical tasar silkworm, *Antheraea mylitta* Drury is important for non-mulberry sericulture. This ecorace is found both in bivoltine and trivoltine forms. The present investigation accounts for some of the antioxidant status in the diapausing pupa of the above two voltine groups. Emphasis was given on the contents of protein, ascorbic acid (Vitamin-C), glutathione (GSH) and level of LPX (MDA content) in the haemolymph and fat body tissues of pupae of the silkworm. Results clearly indicate that the bivoltine cocoons are superior to trivoltine based on their antioxidant status. © 2011 Association for Advancement of Entomology

KEYWORDS: *Antheraea mylitta*, haemolymph, fat body, protein, ascorbic acid, GSH, LPX

INTRODUCTION

All aerobic organisms are endowed with a mutually supportive team of defences against reactive oxygen species (ROS) or the pro-oxidant forces, commonly called as the antioxidant defences. Insects like other animals possess a suite of antioxidant system for the removal of damaging ROS (Felton, 1995). Ascorbic acid is redox catalyst which can reduce, and neutralize ROS such as hydrogen peroxide (Padayatty *et al*., 2003). Glutathione (GSH) is one of the most important cellular antioxidants (Meister and Anderson, 1983). Acute or chronic oxidative stress may result in uncontrolled lipid peroxidation (LPX) and protein oxidation (Levine *et al*., 1981). It has been reported that proteins are vulnerable to oxidative damage (Dean, 1991). This may occur either by interaction between LPX product and proteins or by site specific reactions of protein to ROS. In phytophagous insects, antioxidant defence

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mechanisms are of paramount importance because they are exposed to ROS promotive environments as well as allelochemicals present in the host plants (Sahoo *et al.*, 2008).

Depending upon brood frequency (number of generations produced in a year) the silkworm may be univoltine (UV), bivoltine (BV) and trivoltine (TV) or multivoltine (Morohoshi, 1957). Generally, Daba ecorace of tasar silkworm, *Antheraea mylitta* Drury behaves as BV and TV. A lot of work has been worked out on different aspects of *A. mylitta* D (Jolly *et al.*, 1974; Das and Nayak, 1991; Dey *et al.*, 2010). There is no information about the antioxidant potential of the above ecorace. Usually, the study of antioxidant defence on any commercial animal is important from management point of view, because the antioxidant content influence the chances of survival and multiplying of the animal. So we have studied on some of the antioxidant contents of the pupae of Daba ecorace of tasar silkworm, *Antheraea mylitta* D.

We report herein our analysis on the quantities of proteins, ascorbic acid (ASA), reduced glutathione and level of LPX (MDA content) in the haemolymph (HL) and fat body (FB) tissues of the diapausing pupae of tasar silkworm, *Antheraea mylitta* D.

MATERIALS AND METHODS

The insect

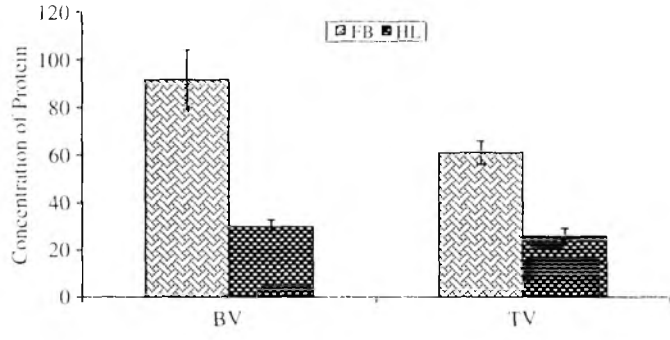
The live bivoltine and trivoltine pupae of Daba ecorace of tasar silkworm, *A. mylitta* were collected time to time from the host plants like Arjun (*Terminalia arjuna*) and Asan (*Terminalia tomentosa*) maintained in the local sericulture rearing field of State Government, Baripada, Mayurbhanj, Orissa.

Tissue preparation

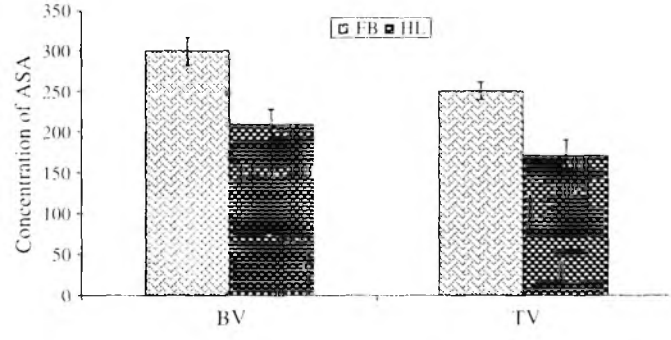
Haemolymph was collected in 3% phenylthiourea coated with prechilled appendorf tube to inhibit denaturing or blackening of the haemolymph (Mishra *et al.*, 2009) by cutting the body segment of pupae and centrifuged at 7,000 × g for 10 minutes at 4°C to settle the haemocytes. Pupae were dissected to collect the fat bodies. After washing in ice-cold physiological saline (0.67% NaCl) the tissues were retained in ice separately. The tissues were weighed in monopan digital balance and homogenized (10%) in 50 mM phosphate buffer pH 7.4 with 1 mM EDTA using hand homogenizer under ice (Sahoo *et al.*, 2008). Homogenates were centrifuged at 10,000 × g for 20 minutes at 4°C. The supernatant were collected for further chemical analyses.

Biochemical estimation

The amount of proteins was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. Ascorbic acid (ASA) content was measured according to the method of Jagota *et al.* (1982). The reduced glutathione (GSH) content in the tissue samples was determined according to the method of Ellman (1959). The sample after centrifugation was used for the estimation of lipid peroxidation (LPX) by monitoring the formation of malondialdehyde (MDA) by the method of Ohkawa *et al.*



FIGURES 1. Concentration of protein in (mg/g) in tissue of HL and FB of BV and TV pupae of *A. Mylitta* D. Data are mean± SD ($n = 8$).



FIGURES 2. Concentration of Ascorbic acid (μg/g) in tissue of HL and FB of BV and TV pupae of *A. Mylitta* D. Data are mean± SD ($n = 8$).

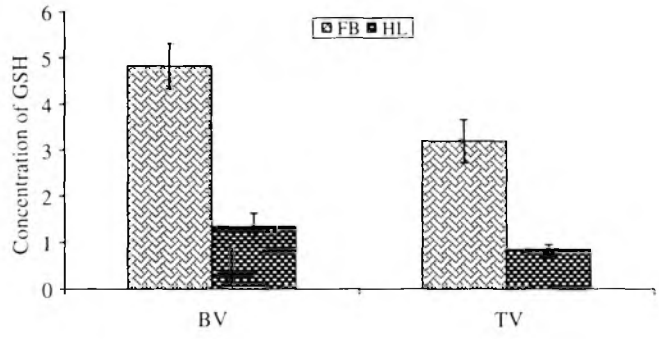
(1979). The amount of MDA formed was calculated from the extinction coefficient of $1.56 \times 10^5 M^{-1}cm^{-1}$ (Wills, 1969).

Statistics

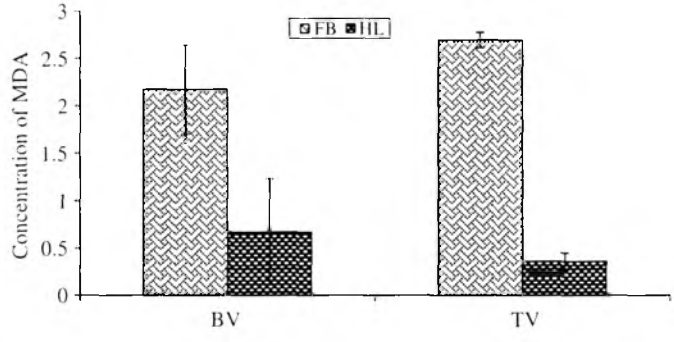
To know the difference between means of two independent samples Fisher's t test and for dependent samples paired t-test were employed (Chainy *et al*., 2008)

RESULTS AND DISCUSSION

Statistical analyses (Fisher t-test) revealed that the concentration of protein, ascorbic acid, GSH and level of LPX (MDA content) of FB was significantly higher ($P < 0.001$) than HL tissue in both BV and TV pupae (Figs. 1-4). This indicates that, the insects preferred to store the antioxidants more in FB rather than HL. As HL is the circulating fluid for its body, their FB becomes store house of antioxidants for future



FIGURES 3. Concentration of GSH (μmol/g) in tissue of HL and FB of BV and TV pupae of *A. Mylitta* D. Data are mean± SD ($n = 8$).



FIGURES 4. Concentration of MDA (malondialdehyde) (μg/g) in tissue of HL and FB of BV and TV pupae of *A. Mylitta* D. Data are mean± SD ($n = 8$).

use in their later stage of life, i.e., moth (adult) stage. The storage of antioxidants in FB is an indication of its requirement during the reproductive period which starts at the end of pupal eclosion.

To compare the antioxidant contents between two voltine of the same tissue, paired t-test was employed. The protein content in FB of BV was significantly higher ($P < 0.001$) than that of the TV (Fig. 1). The HL protein content of BV was also significantly higher ($P < 0.02$) than the TV (Fig. 1). The ASA content of FB was significantly higher ($P < 0.001$) in BV than the TV (Fig. 2). In case of HL the ASA content of BV was also significantly higher ($P < 0.02$) than the TV (Fig. 2). The GSH content found in the FB of BV was significantly higher ($P < 0.001$) than that of the TV (Fig. 3). For HL the GSH content was found statistically higher ($P < 0.01$) in BV than TV (Fig. 3). The MDA formed in the FB tissue of TV was higher but not significant than that of the BV (Fig. 4). The opposite trend is found in HL tissue which showed significantly higher ($P < 0.001$) MDA in BV than TV (Fig. 4).

From the above information it may be concluded that the pupae of BV cocoons were maintaining higher concentration of antioxidants than that of the TV in both FB and HL tissues.

It is observed that the protein, ascorbic acid, GSH and LPX level of different tissues are not same in both the voltine groups. The protein, ascorbic acid and GSH content of the FB and HL was more in BV than TV. The level of formation of MDA in the FB was less in BV than TV and it was opposite in HL. The protein, ascorbic acid, GSH and MDA content was found more in fat body than that of haemolymph except in case of FB of BV where MDA content was less than TV but not significant.

From the present result it can be concluded that the high protein concentration may work as a preventive measures towards oxidative damage in the FB tissue as reported by Dean (1991). The high content of the redox catalyst ascorbic acid in FB indicates higher rate of neutralization of ROS, like hydrogen peroxide in FB than that of the HL. Similarly the high concentration of GSH content in the FB is the indication of cellular antioxidant activity to protect the cell from oxidative damage. Lipids are essential as integral component of cell membrane and particularly for insects these are important as desiccation preventing hydrocarbons and for the synthesis of ecdysone (Downer, 1985). LPX is an extremely potent inhibitor of mitosis and even a low level of peroxidation can slow or totally halt cell cycle progression (Wilber *et al*., 1957; Dianzani, 1993). Comparison of level of LPX in both the tissues of pupae are indicates that FB tissue challenged with relatively higher level of oxidative threat. Considerably higher level of MDA in the FB tissue reflects a relatively high level of activated oxygen species which could be due to their increased production and/or decreased neutralization.

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Identification of a third worker caste in the colony of *Oecophylla smaragdina* (Fabricius) based on morphology and content of total protein, free amino acids, formic acid and related enzymes

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ABSTRACT: The polymorphic colony of *Oecophylla smaragdina* possessed three types of apterous workers such as large (major), small (minor) and a third type intermediates which are smaller than major and larger than minor workers. The body length in millimeter (mean) of major, intermediate and minor were 10.5, 8.3, 4.6 respectively, but they did not exhibit any difference in the shape of antennae, mandibles and in the number of antennal segments where it was always twelve. In minor workers the share of abdomen was 70% of the total body weight, in intermediates it was 50% and in major it was 58%. The worker castes exhibited difference in the content of total protein, free amino acids (FAA) and formic acid (FA). Among the worker castes, intermediate workers possessed highest amount of protein followed by major and minor. The content of FA and FAA exhibited a negative correlation in worker castes. The winged males, females, larvae and pupae possessed elevated levels of both protein and FAA. The workers castes possessed very high activity of both Aspartate amino transferase (AsAT) and Alanine amino transferase (AlAT) which were found to be 10 to 15 times higher than that of developing stages, winged males and females. In apterous workers, winged adults and developing stages of *O. smaragdina*, the ratio of the activity of AlAT with AsAT was always below one, which indicated no twist in metabolism of amino acids during the course of metamorphosis. © 2011 Association for Advancement of Entomology

KEYWORDS: *Oecophylla smaragdina*, free amino acids, formic acid, Aspartate amino transferase, Alanine amino transferase

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INTRODUCTION

Weaver ants are social insects that have a complex chemical communication system. They make elaborate nests out of leaves of trees on which they live. Weaver ants are found in the tropical forests of Africa, Australia and South East Asia. A mature colony of weaver ants can hold as many as 100,000 to 500,000 workers and may span as many as 12 trees and contain as many as 150 nests (Holldobler and Wilson, 1994). They can inflict painful bites which is aggravated by irritating chemicals such as formic acid secreted from the abdomen (Huang and Yang, 1987). The predatory power of *O. smaragdina* is most outstanding among ants in their localities (Way and Khoo, 1992; Peng *et al.*, 1997, 1999; Mele and Cuc, 2000). They can protect the host plants from attacks of phytophagous insects and therefore has been used as pest control agents. Their well-developed system of communication, as well as their caste system enhances the capabilities of the colony, however individual ants are mostly incapable of surviving on their own. The caste system contains different types of apterous workers, winged males, winged females. The developing forms such as egg, larvae and pupae of these adult forms can also be seen among them. The major workers do most of the external work for the colony, including building nests, foraging for food, and exploring new territory. The minor workers are responsible for taking care of the eggs and young larvae (Holldobler and Wilson, 1990). Even though there are many reports by foreign authors that *O. smaragdina* possessed only two types of worker castes we could identify a third category of worker caste with clear difference in size, body proportion and biochemistry which forms subject matter of this publication.

MATERIALS AND METHODS

The ants were collected from the wild with minimum disturbance by putting a medium sized nest in a plastic container which was applied with cotton soaked in chloroform. The mouth of the jar was closed immediately with the lid and after 5 minutes the colony individuals were dropped on a white paper. The different individuals within a nest were separated and their body weight and body length were measured and recorded. For taking the body weight, average weight of 10 individuals (workers) were measured and recorded using an electronic balance with sensitivity of 0.1 mg. The body length of worker ants were determined and photos were taken using calibrated stereo microscope (Leica S8 APO, Germany).

Adults, developing stages (whole body) and body parts of workers were separated, weighed and were subjected to various biochemical quantitative analysis for parameters such as formic acid (Colowick and Kaplan, 1963), total protein (Lowry *et al.*, 1951), total free amino acids (Spies, 1957), and enzymes such as Aspartate amino tranferase (AsAT) and Alanine amino transferase (AlAT) (Reitman and Frankel, 1957). Studies were carried out using the samples collected from various parts of Trivandrum District of Kerala. The estimations were done between the period of June 2009 to June 2010. Each experiment was repeated at a minimum of four and statistical analysis were done as described by Daniel (2006).

TABLE 1. Body length of various individuals of *O. smaragdina* colony

Individuals		Total body length (mm)
Workers	Major	10.5 0.5
	Intermediate	8.3 0.4
	Minor	4.6 0.3
Winged Forms	Males	10.5 0.4
	Females	16.3 0.5

Body length of workers showed signicant difference from one another. P 0 01, n 10.

TABLE 2. Body weight of different worker castes of *O. smaragdina.*

Sample	Major		Intermediate		Minor	
Whole Body	11.8	0.8	6.3	0.4	1.3	0.08
Head	2.3	0.2 (19.5)	1.4	0.09 (22)	0.2	0.02 (15)
Thorax	2.5	0.2 (22.5)	1.9	0.09 (29)	0.2	0.02 (15)
Abdomen	6.7	0.3 (58)	3.4	0.2 (52)	0.9	0.05 (69)

Body weight is expressed in milligrams. All the values of each type of worker are signicantly different from others. P 0 01, n 6.
Values in parenthesis indicate contribution of each body part on percentage basis in relation to the whole body weight.

OBSERVATION AND RESULTS

The observation on an exposed nest revealed that the polymorphic colony of *O. smaragdina* possessed winged males, winged females and three types of apterous workers (major, intermediate and minor). The three types of workers were differing in size but not in shape of the body and the results are shown in the Tables 1 and 2. The intermediate workers are signicantly shorter than major and are almost half of the weight of the major workers. Size ratio of head to abdomen in intermediate workers signicantly differed from that of m ajor and minor workers. At the same time intermediates are almost double the length of minors and four times the weight of minors (Fig. 1a-c). The number of antennal segments in all three types of workers was same and it was 12 segments. Workers are the most abundant individuals in the colony and among them the major workers share was almost 20%, intermediates 70%, and minor 10%.

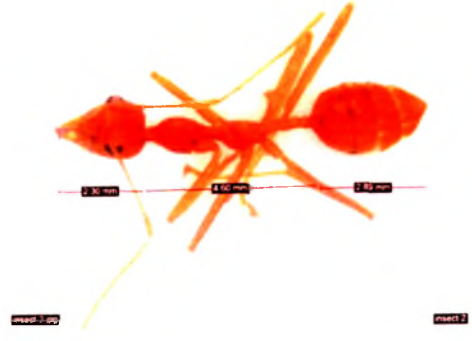
When observed a nest at the onset of south west monsoon (last week of May), a medium sized nest possessed about 206 major workers, 638 intermediates, 92 minor workers, 223 winged males and 14 winged females. The colony structure was not static and it exhibited seasonal variation. The variation in colony structure was reected only in number of eggs, larvae, pupae, adult males and females but the number of worker castes remained almost constant throughout the year. The minor workers were mostly

(a)



Major worker (1x)

(b)



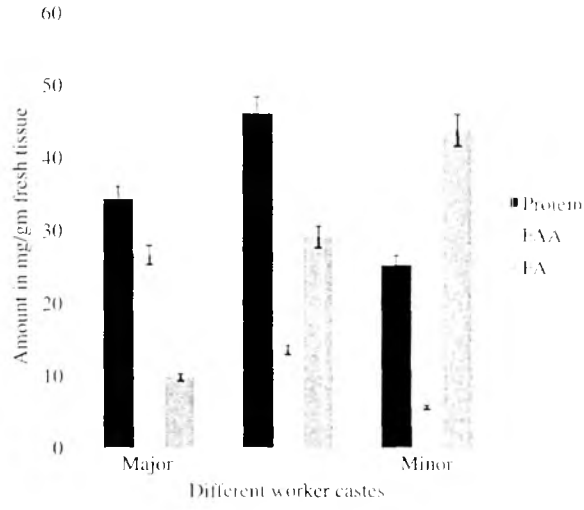
Intermediate worker (1x)

(c)



Minor worker (1.5x)

FIGURES 1. Three different types of worker castes of *O. smaragdina*.



FIGURES 2. Biochemical profile of total protein, free amino acids, and formic acid in the whole body homogenate of different worker castes. Values are expressed in mg/gm fresh tissue. All the values are mean ± SEM, $n = 6$. Values of each parameter is significantly different in Duncan's multiple range tests at 0.05 level.

confined within the nest and major workers were very active in the field as well as in the nest. The intermediate types of workers were detected in the field as well as in the nests. In major worker's contribution of abdomen to the weight of whole body was 60% and in intermediates it was 50% and in minor workers it was 70% (Table 2).

Among the individuals of the colony winged males and larvae possessed very high protein content than the rest of the colony. All the individuals of the colony maintained preferably very high content of FAA. When abdomen, thorax and head of major workers were analyzed the head showed more content of protein than the thorax and abdomen. The amount of FAA in the head and thorax of major worker was almost the same but its content in abdomen was negligibly low (Table 3 and Fig. 2). On comparing the content of the whole body FAA of different worker castes with the content of FA the values exhibit a negative correlation (Table 3 and Fig. 2).

The activity of transaminases, both the Aspartate amino transferase (AsAT) and Alanine amino tranferase (AlAT) showed a very high activity in the thorax and head of worker ants and at the same time the whole body homogenate and the homogenate of abdomen did not show any transaminase activity. The winged males, females and developing stages also exhibited preferably high activity of transaminases (Table 4). In adults and developing stages of *O. smaragdina* the ratio of the activity of AsAT to AlAT was always lower than one.

DISCUSSION

Even though the previous investigators (Holldobler, 1983; Holldobler and Wilson, 1977, 1990; Lokkers, 1986) have reported only two types of workers in the colony

TABLE 3. The content of protein, free amino acids and formic acid in different colony individuals of *O. smaragdina*

Individuals		Protein	Free amino acids	Formic acid
Workers	Major	34.3 ± 2.6	26.6 ± 2.5	9.7 ± 0.7
	Intermediate	46.1 ± 4.2	13.5 ± 1.1	29.1 ± 2.3
	Minor	25.2 ± 2.1	5.5 ± 2.5	43.7 ± 3.1
Major Worker	Head	45.1 ± 2.5	29.2 ± 2.2	0.63 ± 0.02
	Thorax	40.4 ± 2.1	33.1 ± 3.1	0.11 ± 0.01
	Abdomen	17.3 ± 1.2	3.2 ± 0.3	42.9 ± 3.6
Winged Forms	Males	77.7 ± 6.1	51.2 ± 4.7	1.27 ± 0.12
	Females	45.6 ± 2.9	28.9 ± 1.6	1.23 ± 0.11
Developing Forms	Larva	54.5 ± 2.8	23.7 ± 2.1	Nil
	Pupa	46.6 ± 3.1	24.6 ± 2.3	Nil

All values are mean ± SD. $n = 6$. Values are expressed in mg/gm fresh tissue.

TABLE 4. The activity of transaminases in different individuals in the colony of *O. smaragdina*

Sample		Activity of AlAT*	Activity of AsAT**	AlAT/AsAT
Worker (whole body)		Nil	Nil	0
Major Workers	Head	335.3 ± 19.1	500.8 ± 22.6	0.67
	Thorax	414.7 ± 20.6	428.5 ± 20.1	0.96
	Abdomen	Nil	Nil	0
Whole Body	Winged Male	35.4 ± 2.3	56.6 ± 4.1	0.62
	Winged female	34.1 ± 2.4	36.4 ± 2.8	0.94
	Large Worker	26.4 ± 2.2	31.6 ± 2.8	0.83
	Pupa (Worker)	30.8 ± 2.3	42.3 ± 3.1	0.71

All values are mean ± SD. $n = 6$.

*Activity is expressed as nanomoles of pyruvate liberated/min/mg protein.

**Activity is expressed as nanomoles of oxalo acetate liberated/min/mg protein.

of *O. smaragdina* (major and minor), we could identify a third type of worker, an intermediate one which are smaller than major workers and larger than minor workers (Table 1, Fig. 1a–c). On the basis of body proportion the intermediates showed more similarity with major than to minor worker (Table 2). Besides the difference in the body size they exhibited marked variations in content of protein, FAA and FA (Table 3). On the basis of above results it can be convincingly proved that the colony of *O. smaragdina* possessed a third category of workers called as intermediate types which are active in the field as well as in the nest, along with the major workers.

The winged males possessed high amount of proteins among the individuals of colony and they also maintained an elevated level of FAA. The larvae and pupae of

these ants possessed high amount of protein but the content of FAA was only half of the content of protein, which may be due to increased synthesis of protein, or increased turnover of amino acids in developing stages. The larvae and pupae of these ants possessed high amount of proteins, FAA and no traces of FA. It may be reason that the brood of *O. smaragdina* is consumed by tribals of Kerala (Yesodharan *et al.*, 2011) and people of other parts of the world such as Thailand (Holldobler and Wilson, 1994). Among the three types of workers the contents of FAA and FA exhibited a negative correlation. It was reported that FA is produced from free amino acid pool of glycine and histidine (Hefetz and Blum, 1978). The minor workers exhibited highly elevated level of FA through the utilization of FAA pool and hence they possessed low content of FAA when compared with other worker castes (Fig. 2).

The activity of transaminases, both Aspartate amino transferase (AsAT) and Alanine amino transferase (AlAT) in the and thorax of major workers was shown to be very high and which was almost 10 times higher than that of the developing stages such as pupae and larvae (Table 4). This elevation clearly agrees with the physical activity of worker ants which are extremely active than that of larvae and pupae. The homogenate of whole body and the homogenate of abdomen of workers did not show any transaminase activity and it may be due to the presence of strong FA in the abdomen which would have inhibited the enzyme activity during the course of homogenization. The winged males and females also showed high activity of transaminases. The activity of transaminases in other insects such as mosquitoes and their larval forms (Evans and Kaleysaraj, 1992) also exhibited very high activity which is several times higher than the mammalian system (Subramoniam *et al.*, 1998). Elevated level of transaminase activity is the indication of rapid turnover of amino acid for release of energy. In mosquitoes, the larval stages showed a very high activity of both transaminases, which was even higher than that of adult mosquitoes (Evans and Kaleysaraj, 1992), but in *O. smaragdina*, the larval stages showed low activity of transaminases than the adult worker ants because the larval stages of ants are very much inactive than that of mosquito larvae. Keeping the ratios of AlAT with AsAT below one has some biochemical significance. In adult mosquitoes, the ratio of AlAT with AsAT was always above one but in their larval stages the above ratio was always below 0.2 (Evans and Kaleysaraj, 1992). In mosquitoes, the aquatic larvae were metamorphosed in to a blood sucking fly the biochemical machinery within the body would have drastically changed in such a way to adapt the parasitic blood sucking mode of life. In *O. smaragdina* the aerial life of reproductive forms were short and there may not be a drastic change in the food and habitats, and hence the ratio in larvae, pupae and adults were kept as same.

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Ovicidal action of some newer insecticides against eggs of *Eublemma amabilis*—a lepidopteran predator of Indian lac insect, *Kerria lacca* (Kerr)

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ABSTRACT: The lepidopteran predator of lac insect, *Eublemma amabilis* Moore is a serious threat to lac insect culture all over the country where lac cultivation is in practice. Laboratory evaluation of four insecticides viz., indoxacarb, spinosad, fipronil and ethofenprox at different concentrations was carried out for assessing their ovicidal action against one day old eggs of *E. amabilis*. The ovicidal effect of insecticides has been assessed by dipping the egg masses in an insecticidal solution. The insecticides evaluated have shown varying degrees of ovicidal action to *E. amabilis*. The egg inhibition activity amongst different treatments varied from 1.71–67.15% with the treatment of indoxacarb, 36.82–75.44% with fipronil, 81.61–100% with spinosad and 82.02–100% with ethofenprox. Spinosad is a naturalyte compound, produced by fermentation of a rare bacterium, *Sacharopolyspora spinosa*, a synthetic analogue compound have shown very good egg hatching inhibition activity followed by ethofenprox, fipronil and indoxacarb. At the lowest concentration (0.0012% a.i.), the egg mortality was higher than the other insecticides evaluated. The recommended concentration of spinosad (0.005% a.i.), indoxacarb (0.007% a.i.), fipronil (0.007% a.i.) and ethofenprox (0.02% a.i.) based on field application against three major predators namely *E. amabilis*, *Pseudohypatopa pulverea* (Lepidopteran) and *Chrysopa* spp. (Neuropteran) of lac insect exerted 100, 50.9, 61.5 and 84.6% egg hatching inhibition activity against eggs of *E. amabilis* under laboratory condition. Application of these insecticides will be very much effective in reducing the incidence of *E. amabilis* if applied at proper time. Our findings indicate that spinosad and fipronil with low active ingredients and high mortality could be the best option for suppressing the incidence of *E. amabilis* in the egg stage.
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KEYWORDS: Ovicidal action, insecticides, *Eublemma amabilis*, *Kerria lacca*

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INTRODUCTION

The Indian lac insect, [*Kerria lacca* (Kerr)] (Homoptera: Tachardiidae) is one of important productive insect secreting products of commercial importance namely, resin, wax and lac dye which finds application in several industries. Lac insect thrives on more than 400 host plants but *Schleichera oleosa (kusum), Ziziphus mauritiana (ber), Butea monosperma (palas) and Flemingia semialata* are the major lac host plants on which lac insect reared commercially. Lac cultivation is one of the major sources of livelihood for poor resource constraint people mainly tribal inhabiting in forest and sub-forest areas of Jharkhand, Chhattisgarh, Madhya Pradesh, West Bengal, Odisha, Andhra Pradesh, Assam and Uttar Pradesh states of India. In today's perspective, lac cultivation is one of the best ecological approaches for economic development of forest and sub-forest dwellers where lac host plants are available abundantly. By creating awareness among the forest and sub-forest dwellers about the importance of available natural resources in their livelihood support, the aim of conserving forest as well as environment can be achieved to a great extent. The production of lac is subject to risk by abiotic and biotic factors and without managing them the sustainable yield cannot be ensured. The biotic factors include insect-predators, parasites and pathogens. The lac crop is attacked by a number of predators and parasitoids but the lepidopteran predators mainly *Eublemma amabilis* Moore (Lepidoptera: Noctuidae) and *Pseudohypatopa pulverea* Meyr (Lepidoptera: Blastobasidae) are intimately associated with lac culture causing around 40% damage to lac crop annually (Glover, 1937; Malhotra and Katiyar, 1979). Besides several cultural practices, chemical control measures for managing these lepidopteran pests have also been evolved. Endosulfan (Malhotra and Katiyar, 1975, 1979), ethofenprox (Jaiswal *et al.*, 2004) dichlorvos (Mishra *et al.*, 1995, 1996) and diflubenzuron (Bhattacharya *et al.*, 1997) are some recommended insecticides which are safe to lac insect and are effective in management of the lepidopteran pests, if applied at proper time and concentration. Egg stage is one of the most vulnerable stages in life cycle of an insect. Targeting this stage will be more relevant and fruitful particularly in case of borer pests and the use of insecticidal application as well as pesticide load in an ecosystem can be minimized. Larvae of *E. amabilis* penetrating into lac encrustation just after hatching from the eggs and complete its larval and pupal periods inside the lac encrustation. Keeping that thing in mind, four insecticides *viz.*, indoxacarb, spinosad, fipronil and ethofenprox which are safe to lac insects and effective in suppressing the incidence of this predator under field condition have been evaluated for their ovicidal action against eggs of *E. amabilis* under laboratory conditions.

MATERIALS AND METHODS

For assessing the ovicidal activity of insecticides against eggs of *E. amabilis*, the egg masses were obtained from laboratory cultured adults of *E. amabilis*. The adult stages of *E. amabilis* have been collected from used-up broodlac sticks (*phunki*) of summer season *kusmi* lac crop derived from tree of *S. oleosa* during August 2011. These lac sticks were kept in wooden cages, fitted with glass tubes on one side of the box. The

collected adult stages (male and female) were transferred into egg laying chamber prepared with two liter plastic container covered with muslin cloth at the top end for better aeration. Honey solution, soaked in cotton was provided to adult as feed. The female lays eggs in cluster on paper stripes kept in egg laying chamber. The method for assessing ovicidal activity was similar to leaf dip method used by Tabashnik and Cushing (1987) against *Plutella xylostella*. Known numbers of one-day old eggs were dipped for two minutes in different concentrations of freshly prepared insecticidal solution with the help of forceps. Dipped eggs were then immediately kept under fan on glass Petri dish for drying. The Petri dish was covered after 2--3 hrs when paper strips bearing egg masses were dried. In control, only water has been used for dipping of eggs. The commercial formulation of insecticides available in the market *viz.*, indoxacarb 14.8% EC (Dhawa Gold®from Dupont India Pvt Ltd); spinosad 2.5% SC (Success®from Dow Agrosciences, India Ltd); fipronil 5% SC (Regent®from Bayer Crop Science) and ethofenprox 10% EC (Nukil®from Dhanuka Pesticides Limited, India) were procured from the market and were used in the present study. Five concentrations of each insecticide *viz.*, indoxacarb (0.0017, 0.0035, 0.007, 0.014 and 0.021% a.i.), fipronil (0.0012, 0.0025, 0.005, 0.007 and 0.01% a.i), spinosad (0.0012, 0.0025, 0.005, 0.007 and 0.01% a.i.) and ethofenprox (0.005, 0.01, 0.02, 0.03 and 0.04% a.i.) were evaluated. There were nine replicates for each concentration of insecticides having varying number of eggs. The observations on number of hatched and unhatched eggs were recorded daily till the emergence is completed. The corrected per cent unhatched eggs with respect to control were calculated by ABBOTT'S formula (1925).

RESULTS AND DISCUSSION

Perusal of data presented in Table 1 indicates differential ovicidal activity in different treatments. The inhibition of egg hatching (corrected percentage) was recorded to the tune of 1.71 to 67.15% with the treatment of indoxacarb, 36.82 to 75.44% with fipronil, 81.61 to 100% with spinosad and 82.02 to 100% with ethofenprox (Table 1). The treatment with indoxacarb showed significant difference in hatching inhibition over control in all the concentrations evaluated except 0.0017% a.i., which was lowest and at par with the control. In respect of indoxacarb, 0.022 and 0.014% a.i. concentrations have been recorded as the best treatment causing 67.59 and 53.74% hatching inhibition respectively over control. The next group of indoxacarb treatments which differ significantly from the above treatments is 0.014 and 0.007% a.i. concentrations causing 53.74 and 50.57% egg hatching inhibition, respectively over control. Indoxacarb (0.007% a.i.) causes significantly higher hatching inhibition than half of its concentration (0.0035 % a.i.). Though the indoxacarb @ 0.021% a.i. concentration causes significantly higher hatching inhibition than 0.007% a.i. but the hatching inhibition response was not encouraging with respect to dose which is about three times higher than that of former. The estimated concentration of indoxacarb which causes 50% egg hatching inhibition comes to 0.013% a.i.. All the concentrations of fipronil tried showed significant reduction in hatching over control.

Four concentrations 0.0025, 0.005, 0.007 and 0.01% a.i. of fipronil were found to be equally effective in which egg hatching inhibition varied from 67.76 to 76.53%. The value of estimated concentration which causes 50% inhibition of hatching comes to 0.004% a.i.. Treatment with spinosad have shown very good ovicidal action against *E. amabilis* and cent per cent hatching inhibition was recorded with the treatment of spinosad @ 0.005, 0.007 and 0.01% a.i. concentrations. Even the lowest concentration of spinosad (0.0012% a.i.) tried have shown 81.90% egg hatching inhibition over control. The egg hatching inhibition was recorded to the tune of 94.96% over control with the treatment of spinosad @ 0.0025% a.i. and significantly higher than the lowest concentration evaluated. Treatment with ethofenprox was also found to be very much effective against eggs of *E. amabilis* where hatching inhibition in different treatments varied from 83.33 to 100% over control. A significant difference in inhibition of egg hatching over control was recorded even at lowest concentration (0.005% a.i.) of ethofenprox evaluated for their ovicidal action. Concentration of 0.03 and 0.04% a.i. of ethofenprox exerted 92.55 and 100% egg hatching inhibition effect respectively over control. The present study indicated that all the four insecticides evaluated exhibited ovicidal action on eggs of *E. amabilis*.

The recommended concentration of indoxacarb (0.007% a.i.), fipronil (0.007% a.i.), spinosad (0.005% a.i.) and ethofenprox (0.02% a.i.) based on field evaluation for their safety to lac insect and efficacy against predators of lac insect exerted 50.91, 61.50, 100.00 and 84.95% inhibition of egg hatching (corrected) of *E. amabilis*, respectively. Based on ovicidal action of these insecticides, it can be inferred that spinosad was found to be best treatment followed by ethofenprox, fipronil and indoxacarb. The egg stage is available under field as soon as larval settlement is over as the emergence of adult moths of *E. amabilis* takes place during that period (1st week of July, 4th week of August, 2nd week of October and February). This stage is better exposed to external environment in comparison to larvae which bores the encrustation and hide under it. Hence, lower incidence of this predator due to application of these insecticides on standing lac crop ((Singh *et al.*, 2011) personal observation) might be attributed due to their ovicidal action also.

Our findings with respect of ovicidal action of these insecticides are in conformity with the findings of earlier workers. Good ovicidal activity of fipronil and spinosad against *Plutella xylostella* (L.) has been reported by Mohammad Mahmoundv *et al.* (2011). Charmillot *et al.* (2007) have reported the ovicidal action of spinosad and indoxacarb against *Grapholita lobarzewskii*. Ovicidal action of spinosad on European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae) have been studied and reported by Boiteau and Noronha (2007). Whereas ovicidal activity of ethofenprox against eggs of *Spodoptera litura* have been reported by Peter and David (1990).

Bhattacharya *et al.* (1995) evaluated and reported the ovicidal action of diflubenzuron against the eggs of *E. amabilis*. The exposure of UV radiation on eggs of *E. amabilis* also indicated inhibition of egg hatching and LT_{50} and LT_{90} values have been found to be 9, 18 minutes and 21, 45 minutes, respectively (Bhattacharya *et al.*, 1998).

TABLE 1. Ovicidal action of different insecticides on *Eublemma amabilis* eggs exposed to various concentrations of commercial formulation

Indoxacarb (14.8 % EC)				Fipronil (5% EC)				Spinosad (2.5% EC)				Ethofenprox (10% EC)			
Conc (%)	Total number of eggs treated	% of hatching inhibition	Corrected % of hatching inhibition	Conc (%)	Total number of eggs treated	% of hatching inhibition	Corrected % of hatching inhibition	Conc (%)	Total number of eggs treated	% of hatching inhibition	Corrected % of hatching inhibition	Conc (%)	Total number of eggs treated	% of hatching inhibition	Corrected % of hatching inhibition
0.021	540	67.59^{e} (365)	67.15	0.01	1095	76.53^{e} (838)	75.44	0.01	900	100.00^{e} (900)	100.00	0.04	900	100.00^{e} (900)	100.00
0.014	428	53.74^{de} (230)	53.10	0.007	983	74.26^{de} (730)	73.07	0.007	900	100.00^{de} (900)	100.00	0.03	832	92.55^{de} (770)	91.96
0.007	413	51.57^{de} (213)	50.91	0.005	916	63.21^{cde} (579)	61.50	0.005	900	100.00^{cde} (900)	100.00	0.02	774	86.05^{cde} (666)	84.95
0.0035	207	26.09^{bcd} (54)	25.07	0.0025	912	67.76^{cde} (618)	66.27	0.0025	855	94.96^{abcd} (812)	94.87	0.01	758	84.30^{abcd} (639)	83.07
0.0017	230	3.04^{ab} (7)	1.71	0.0012	583	39.62^{bc} (231)	36.82	0.0012	737	81.90^{bc} (604)	81.61	0.005	750	83.33^{abcde} (625)	82.02
Control	220	1.36^{a} (3)	0.00	Control	945	4.44^{a} (42)	0.00	Control	900	1.85^{a} (17)	0.00	Control	810	7.28^{a} (59)	0.00
df		5				5				5				5	
F		30.87				13.61				219.99				173.35	
P		0.05				0.05				0.05				0.05	

Figures in parentheses indicate absolute values of unhatched eggs

Figures (%) marked with different letters of superscript within same column are significantly different ($P<0.05$)

The study indicated that the application of these insecticides at the time of egg laying stage of the lac insect predator, *E. amabilis* will be useful in nipping the problem of incidence in early stage itself. These newer insecticides can be suitably integrated in pest management programme of lac ecosystem for containing the menace of this important predator of Indian lac insect without much disturbing the environment.

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Efficacy of pyrethroid based insecticides against *Rhizoecus amorphophalli* infested tubers of *Amorphophallus paeoniifolius* under laboratory conditions

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ABSTRACT: Elephant foot yam, *Amorphophallus paeoniifolius* is an edible aroid widely cultivated in the tropical countries. On harvest, the tubers are either marketed directly as vegetable or stored as seed materials. Infestation by the mealy bug, *Rhizoecus amorphophalli* is a serious problem during its long-term storage. This sap sucking insect pest attacks the stored tubers and sucks the juice leading to desiccation and shriveling of tubers. Mealy secretions from the insect disfigure the tubers affecting their acceptability and marketability. Proper management of this noxious pest plays a crucial role in avoiding the storage damage inflicted by the mealy bugs. Present study aims to check the efficacy three pyrethroid insecticides against this mealy bug under laboratory conditions. All the 1st instars sprayed with lambda cyhalothrin 0.01% were completely killed, whereas 0.05% of cypermethrin and fenvalerate was required to same mortality on the third day after treatment. Cypermethrin and lambda cyhalothrin at 0.5% was found effective for the control of adult females but other fenvalerate made only 86.66% mortality on the 3rd day and proved to be less effective. Dipping the mealy bug infested tubers in cypermethrin and lambda cyhalothrin at 0.5% produced complete control over this pest on the first day itself. Therefore, dipping the infested tubers for ten minutes in any of these insecticides can be recommended for the management of this pest. For achieving better results, the strategy has to be aimed at controlling the establishment of young crawlers by dipping the tubers for several times until crawler production ceases, thereby controlling the mealy bug population.
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KEYWORDS: pyrethroid insecticides, *Rhizoecus amorphophalli*, *Amorphophallus paeoniifolius*

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INTRODUCTION

Insect pests have been a menace to man since they started cultivating and often severe pest out breaks minimize the crop production and reduce agricultural outcome. Climate change has paved the way for transformation of hitherto known minor pests to the status of major pests especially the mealy bugs (Homoptera: Pseudococcidae) which are highly polyphagous and gregarious in nature (Suresh and Kavitha, 2008). Being a sucking pest, they feed on plant juices from most agricultural crops including the stored products and also act as a vector of several plant diseases (Ben-Dov, 1994).

Genus *Rhizoecus*, encompass more than 120 species of world widely reported root mealy bugs having a wide host range (Williams, 1985; Danzig *et al.*, 2008). *Rhizoecus amorphophalli* Betrem is the noxious pest infesting the stored tubers of major yams and aroids especially elephant foot yam (Palaniswami, 1994; Rajamma *et al.*, 2002). They are white, slow-moving, oval, soft bodied insects with a white waxy coating over their body.

Elephant foot yam, *Amorphophallus paeoniifolius* (Dennst.) Nicolson is an edible aroid cultivated in the tropical countries and in India it is traditionally cultivated in West Bengal, Andhra Pradesh, Tamil Nadu, Bihar, Gujarat, Kerala and Jharkhand (Nedunhezhiyan, 2008) with a production potential of 50–80 t/ha (Ravi *et al.*, 2009). The tubers are rich in starch and a good source of minerals and vitamins, therefore used as a vegetable after cooking (Misra *et al.*, 2002). Moreover, many indigenous ayurvedic and unani medicinal preparations were also made using these tubers (Srinivas and Ramanathan, 2005). On harvest, the tubers were either marketed or stored as seed materials for the next planting season and the mealy bug infestation is prevalent during this storage period. They suck and desap the cell content of the tubers and severely infested tubers shriveled, adversely affecting their cooking quality (Palaniswami, 1994). In addition, mealy substance secreted by this insect disfigures the tuber, which affects their acceptability and marketability (Hegde and Jayaprakas, 2011).

Initially, the infestation cannot be noticed as the crawlers are microscopic, but while infestation develops, colonies build up quickly and consequently the tuber will be totally covered by this insect. Relentless infestation results in vast crop failure and consequently severe economic loss to the farmers. Large quantities of stored tubers were rendered unfit for human consumption as a result of insect attack (Adesuyi, 1973). Even though the intensity of infestation by this pest and its economical loss to farmers are indisputable, not much work has been reported on their management. Even though pesticides are perilous to human health and their environment, mealy bug outbreaks require the use of insecticides due to the rapid multiplication of this insect when compared with other pests (Bartlett and Clancy, 1972). Adverse effect of insecticide treatments on the mealy bug population density is characterized by the frequent use of non-selective insecticides (Franco *et al.*, 2004) and non-timely application of these chemicals. The present study aims to screen the efficacy of six locally available pyrethroid insecticides on the mealy bug *R. amorphophalli* under laboratory conditions.

MATERIALS AND METHOD

1. Maintenance of insect culture

Medium sized tubers of elephant foot yam, *A. paeoniifolius* (700–2000 g), totally devoid of any insect infestation were selected and procured from farm office, Central Tuber Crop Research Institute (CTCRI), Sreekariyam. These tubers were cleaned, washed with water and shade dried for 4–5 hours. The adult females of amorphophallus mealy bug, *R. amorphophalli* was obtained from the mother culture maintained on tubers of elephant foot yam in the Bio pesticide laboratory, CTCRI and insects were transferred to the uninfested tubers using a sable haired brush for maintaining pure culture for laboratory assay. These tubers were kept inside wooden cages (60 cm × 60 cm × 60 cm) having the door in the front and other sides covered with wire mesh supplied with a 15-W fluorescent light. The insect culture was transferred to the fresh set of tubers according to the decay or damage of infested tubers.

2. Preparation of insecticidal solutions

Pyrethroids – cypermethrin (Ustaad 10% EC), lambda cyhalothrin (Reeva, 5% EC), fenvalerate (Arfen, 20% EC) were purchased, and diluted to 0.50, 0.10, 0.05, 0.01, 0.005 and 0.001% for the assay.

3. Treatment method

First instars (freshly emerged crawlers) and adult females were carefully collected from the tubers with the aid of sable haired brush and were transferred to separate petri dishes. Freshly prepared insecticidal solutions were taken in atomiser and sprayed on the insects.

In another experiment, scoops having 3 cm^3 diameter were taken from the fully infested tubers using cork borer. These scoops were observed under stereo zoom binocular microscope (Leica M10, Leica Microsystems, Wetzar, Germany – magnification of 50×) to confirm that these scoops contain a good population of live insects. These scoops were dipped for 10 minutes in the freshly prepared insecticidal solutions taken in a 100 ml plastic container. Then using forceps these pieces of tubers were transferred to filter paper kept in small petridishes so that the excess solution was removed. Three replicates were maintained and water was used as control in both experiments.

4. Observation of mortality

Under the microscopic view, mortality count was observed continuously for three days after treatment (1, 2, and 3 DAT). For the dipping method the mortality percentage was calculated as follows

Mortality percentage (%) = (Number of dead insects ÷ number of live insects) × 100

5. Statistical analysis

Statistical analysis was done by Analysis Of Variance (ANOVA) on randomly block design and pair wise comparison was carried out by Duncan's Multiple Range Test (DMRT; $p = 0.05$) using SPSS 17.0.

6. Effect of pyrethroid insecticides on *Rhizoecus amorphophalli*

One day after application, cent percent mortality of first instar was observed in the treatments with all the selected insecticides at conc. 0.50% (Table 1). Complete mortality of first instars was observed due the treatment of cypermethrin and lambda cyhalothrin even at 0.05%, whereas, fenvalerate at the same concentration killed only 90%, and no mortality was observed in control batch. Lambda cyhalothrin and fenvalerate at 0.001% recorded 90.0% mortality, whereas cypermethrin at this conc gave significantly less mortality (76.6%) than other treatments.

On second day, mortality of treatment with fenvalerate at conc. 0.10 and 0.05% increased to 100% (Table 2). Fenvalerate at 0.01% conc. also showed increase in mortality (90.00–96.66%). Cypermethrin at 0.001% was significantly least effective (80.00%) among the insecticidal treatments.

Third day after application, cent percent mortality of first instar was observed in the treatments with all the selected insecticides at conc. 0.05% (Table 3). Cypermethrin at 0.01% conc. also recorded 100% mortality which was *on par* with fenvalerate at the same concentration. All other treatments recorded similar mortality except cypermethrin at conc. 0.001% which gave the least effect (80.0%). No mortality was observed for the control batches throughout the observation period.

In the case of adults, treatment with cypermethrin and lambda cyhalothrin at the conc of 0.5% caused 100% mortality on one day after application (Table 3). lambda cyhalothrin at 0.1% also recorded cent percentage mortality. Fenvalerate (0.5%) gave 86.66% mortality which was statistically similar to 0.1% cypermethrin. All other treatments recorded similar mortality ranging from 70.00 to 46.66%. The lowest mortality (33.33%) with the 0.001% concentration was recorded with the treatment with fenvalerate.

On the second day, no significant increase in mortality of adult mealy bugs was observed in all insecticidal treatments (Table 5). Mortality increased from 46.7 to 50.0% in treatment of cypermethrin (0.005%) and from 56.7 to 60.0% in lambda cyhalothrin at 0.01%.

There was no increase in mortality after the second day and third day observation (data not shown) recorded similar mortality percentage as the second day.

Dipping of mealy bug infested tubers in 0.5% of cypermethrin and lambda cyhalothrin provided cent percentage effect in 1 DAT itself (Table 6). There was no increase in mortality according to days in all the insecticidal treatments. Cypermethrin at 0.10% recorded 89.41% which was *on par* with mortality at conc. 0.50%. Similarly mortality at 0.05% was 79.33% which was statistically similar to treatment with 0.10%. Cypermethrin at lower concentrations (0.01, 0.005 and 0.001%)

TABLE 1. Mortality (%) of 1st instars of mealy bug one day after treatment

Insecticides	Mortality, (%)						
	0.50%	0.10%	0.05%	0.01%	0.005%	0.001%	Control
Cypermethrin	100.00±0.00^{e}	100.00±0.00^{e}	100.00±0.00^{e}	90.00±0.00^{d}	83.33±5.77^{c}	76.66±5.77^{b}	0.0±0.00^{a}
Lambda cyhalothrin	100.00±0.00^{e}	100.00±0.00^{e}	100.00±0.00^{e}	90.00±0.00^{d}	90.00±0.00^{d}	90.00±0.00^{d}	0.0±0.00^{a}
Fenvalerate	100.00±0.00^{e}	96.66±5.77^{c}	90.00±0.00^{d}	90.00±0.00^{d}	90.00±0.00^{d}	90.00±0.00^{d}	0.0±0.00^{a}

Mean with the same letter are not statistically significant by Duncan's multiple range test

TABLE 2. Mortality (%) of 1st instars of mealy bug two day after treatment

Insecticides	Mortality, (%)						
	0.50%	0.10%	0.05%	0.01%	0.005%	0.001%	Control
Cypermethrin	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{d}	90.00±0.00^{c}	83.33±5.77^{b}	80.00±0.00^{b}	0.0±0.00^{a}
Lambda cyhalothrin	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{d}	90.00±0.00^{c}	90.00±0.00^{c}	90.00±0.00^{c}	0.0±0.00^{a}
Fenvalerate	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{d}	96.66±5.77^{d}	90.00±0.00^{c}	90.00±0.00^{c}	0.0±0.00^{a}

Mean with the same letter are not statistically significant by Duncan's multiple range test

TABLE 3. Mortality (%) of 1st instars of mealy bug three day after treatment

Insecticides	Mortality, (%)						
	0.5%	0.1%	0.05%	0.01%	0.005%	0.001%	Control
Cypermethrin	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{d}	90.00±0.00^{c}	83.33±5.77^{b}	80.00±0.00^{b}	0.0±0.00^{a}
Lambda cyhalothrin	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{c}	90.00±0.00^{c}	90.00±0.00^{c}	0.0±0.00^{a}
Fenvalerate	100.00±0.00^{d}	100.00±0.00^{d}	100.00±0.00^{d}	96.66±5.77^{d}	90.00±0.00^{c}	90.00±0.00^{c}	0.0±0.00^{a}

Mean with the same letter are not statistically significant by Duncan's multiple range test

TABLE 4. Mortality (%) of adult females one day after treatment

Insecticides	Mortality, (%)						
	0.5%	0.1%	0.05%	0.01%	0.005%	0.001%	Control
Cypermethrin	100.00 ± 0.00^{h}	93.33 ± 5.77^{gh}	66.66 ± 5.77^{ef}	60.00 ± 10.00^{cdef}	46.66 ± 5.77^{c}	50.00 ± 10.00^{cd}	0.0 ± 0.00^{a}
Lambda cyhalothrin	100.00 ± 0.00^{h}	100.00 ± 0.00^{h}	70.00 ± 0.00^{f}	56.66 ± 5.77^{cdef}	53.33 ± 11.54^{cde}	46.66 ± 5.77^{c}	0.0 ± 0.00^{a}
Fenvalerate	86.66 ± 5.77^{g}	63.33 ± 5.77^{def}	53.33 ± 20.54^{cde}	50.00 ± 10.00^{cd}	46.66 ± 5.77^{c}	33.33 ± 5.77^{b}	0.0 ± 0.00^{a}

Mean with the same letter are not statistically significant by Duncan's multiple range test

TABLE 5. Mortality (%) of adult females two days after treatment

Insecticides	Mortality, (%)						
	0.5%	0.1%	0.05%	0.01%	0.005%	0.001%	Control
Cypermethrin	100.00 ± 0.00^{h}	93.33 ± 5.77^{gh}	66.66 ± 5.77^{ef}	60.00 ± 10.00^{cdef}	50.00 ± 0.00^{c}	50.00 ± 10.00^{cd}	0.0 ± 0.00^{a}
Lambda cyhalothrin	100.00 ± 0.00^{h}	100.00 ± 0.00^{h}	70.00 ± 0.00^{f}	60.00 ± 0.00^{cdef}	53.33 ± 11.54^{cde}	46.66 ± 5.77^{c}	0.0 ± 0.00^{a}
Fenvalerate	86.66 ± 5.77^{g}	63.33 ± 5.77^{def}	53.33 ± 20.54^{cde}	50.00 ± 10.00^{cd}	46.66 ± 5.77^{c}	33.33 ± 5.77^{b}	0.0 ± 0.00^{a}

Mean with the same letter are not statistically significant by Duncan's multiple range test

TABLE 6. Mortality (%) of mealy bugs on the infested tubers dipped in insecticides

Conc. (%)	Cypermethrin			Lambda cyhalothrin			Fenvalerate		
	1 DAT	2 DAT	3 DAT	1 DAT	2 DAT	3 DAT	1 DAT	2 DAT	3 DAT
0.50	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{e}	100.00 ± 0.00^{e}	100.00 ± 0.00^{e}	79.56 ± 5.42^{e}	79.56 ± 5.42^{e}	79.56 ± 5.42^{e}
0.10	89.41 ± 18.44^{cd}	89.41 ± 18.44^{cd}	89.41 ± 18.44^{cd}	78.31 ± 8.82^{d}	78.31 ± 8.82^{d}	78.31 ± 8.82^{d}	64.90 ± 16.42^{de}	64.90 ± 16.42^{de}	64.90 ± 16.42^{de}
0.05	79.33 ± 9.00^{c}	79.33 ± 9.00^{c}	79.33 ± 9.00^{c}	75.12 ± 8.22^{cd}	75.12 ± 8.22^{cd}	75.12 ± 8.22^{cd}	65.71 ± 7.74^{de}	65.71 ± 7.74^{de}	65.71 ± 7.74^{de}
0.01	57.82 ± 8.42^{b}	57.82 ± 8.42^{b}	57.82 ± 8.42^{b}	61.42 ± 6.64^{bc}	61.42 ± 6.64^{bc}	61.42 ± 6.64^{bc}	52.50 ± 14.54^{cd}	52.50 ± 14.54^{cd}	52.50 ± 14.54^{cd}
0.005	41.84 ± 13.10^{b}	41.84 ± 13.10^{b}	41.84 ± 13.10^{b}	57.20 ± 4.82^{b}	57.20 ± 4.82^{b}	57.20 ± 4.82^{b}	45.14 ± 3.60^{bc}	45.14 ± 3.60^{bc}	45.14 ± 3.60^{bc}
0.001	42.54 ± 10.92^{b}	42.54 ± 10.92^{b}	42.54 ± 10.92^{b}	50.52 ± 23.52^{b}	50.52 ± 23.52^{b}	50.52 ± 23.52^{b}	32.44 ± 9.21^{b}	32.44 ± 9.21^{b}	32.44 ± 9.21^{b}
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Mean with the same letter are not statistically significant by Duncan's multiple range test

recorded similar mortality. Fenvalerate was proved to be least effective among the insecticides as they recorded only 79.56% mortality even at 0.50% conc. Control batches recorded no mortality in the treatments.

DISCUSSION

Compared with organophosphate insecticides, those belonging to pyrethroid group were less employed in management of mealy bugs. But, pyrethroid insecticides such as ethion, zeta cypermethrin, bifenthrin, fenvalerate were being used by farmers for mealy control in varied crops (Ahmad *et al.*, 2011). Present study revealed that lambda cyhalothrin at 0.001% killed 100% of 1st instars on 3 DAT, whereas cypermethrin and fenvalerate at 0.05% were required to reach the similar effect. Treatment with fenvalerate produced least effect among the insecticides treated, but this result defers with the findings of (Dhingra, 1990) who proved fenvalerate also equally effective against *D. mangifera*.The variation in species might be the reason for its non effectiveness against yam mealy bug. Persad and Khan (2000) reported that *M. hirsutus* can effectively be controlled using lambda cyhalothrin, but Nagrare *et al.* (2011) recommended cypermethrin and fenvalerate for the control of cotton mealy bug. Even though fenvalerate has been recommended for other mealy bugs, present study proved that it was least effective among the three insecticides tested for control of *R. amorphophalli*.

Present study revealed that high concentrations of insecticides were required to kill the adult females than the 1st instars, and this result supports the findings of Zettler (2002) from the dose response tests of different insecticides against *M. hirsutus*. They observed that adult mealy bugs were more tolerant than the early nymphs. Persad and Khan (2000) and Yousuf *et al.* (2007) also opined that it is difficult to obtain satisfactory control of adult females and the larger nymphs of *M. hirsutus* with contact insecticdes.

Patel *et al.* (2010) reported that the efficacy of buprofezin against *P. solenopsis* on cotton was found to be dose dependent. Similarly, all insecticidal treatment against *R. amorphophalli* recorded an increase or decrease according to the concentration of the pesticides used; mortality decreased as the concentration of insecticides decreased. Insecticides can kill the mealy bugs those are seen on the surface of the plant, but those are seeking shelter in the crevices of the bark will escape from the insecticidal spray, and the residual population can quickly rebuild their colony and cause serious damage (Manjunath , 1985). High volume application of insecticides is advised to ensure wide coverage to kill the mealy bugs that are seen in crevices and cracks on the bark (Lakra *et al.*, 1980). While dipping the mealy bug infested tubers in the recommended insecticides, care has to be taken that tuber area gets fully covered by insecticidal solutions.

Khushk and Mal (2006) worked on the effect of chemical pesticides on the mealy bugs infesting cotton plant and recommended profenofos and chorpyrifos at a dose of 1 litre/acre for satisfactory control up to five days. They also opined that a second application is needed to suppress the mealy bug incidence at a maximum level. The

architecture of elephant foot yam tuber provides congenial atmosphere for mealy bugs to hide, hence repeated application of insecticides is the only solution to dispel the residual population. In case of mealy bugs on elephant foot yam, even though most insecticides were able to suppress the population to a maximum level, repeated application should be done according to the emergence of crawlers, if any. Therefore, establishment of young mealy bugs has to be prevented by applying insecticides several times until crawler production ceases (Martin and Workman, 1999). Tanwar *et al.* (2010) recommended repeated use of the same chemical insecticide may be avoided as there are chances for development of resistance in the pest.

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Effects of frontal ganglionectomy on feeding and maxillar morphology of the final instar larvae of *Oryctes rhinoceros* (Coleoptera: Scarabaeideae)

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ABSTRACT: The functional role of frontal ganglion, associated with stomatogastric nervous system in *Oryctes rhinoceros*, was studied by ganglionectomy. The frontal ganglionectomised larvae of *Oryctes rhinoceros* showed an immediate cessation of feeding and loss of mobility of mouth parts. The scanning electron micrographs of maxilla revealed that, in the larvae of *Oryctes rhinoceros*, the morphology and number of chemosensory sensilla are affected by the frontal ganglionectomy. Degeneration of the trichoid and basiconic sensilla may result in a weakened chemostimulatory input from the maxillary sensory system causing cessation of feeding. The frontal ganglionectomised larvae also showed a permanent arrest of gut movements leading to reduced and desiccated faecal output. Further, it was noticed that frontal ganglionectomy in *Oryctes rhinoceros* larvae caused impairment of metamorphosis subsequently leading to 100% mortality.

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KEYWORDS: *Oryctes rhinoceros*, Stomatogastric nervous system, frontal ganglionectomy, maxillary sensilla

Sensory system in insects has evolved numerous specialisations to permit them to detect features of external environment and to monitor constantly the internal state of an organism. Food selection and gustation are of primary importance to sustain growth and development in voracious immature stages (Zacharuk and Shields, 1991). In most insects, among the external organs associated with feeding, maxilla is the primary sensory structure with virtual tasting capability. This property of maxilla is due to the presence of variously modified integumentary processes with rich nerve supply known as sensilla. The present study gives an account of the effect of frontal ganglionectomy on feeding as well as on the morphology of maxillary sensilla in the final instar larvae of *Oryctes rhinoceros*.

Healthy final instar larvae of *Oryctes rhinoceros*, collected from the manure pits of Thiruvananthapuram, were reared in the laboratory following the method of

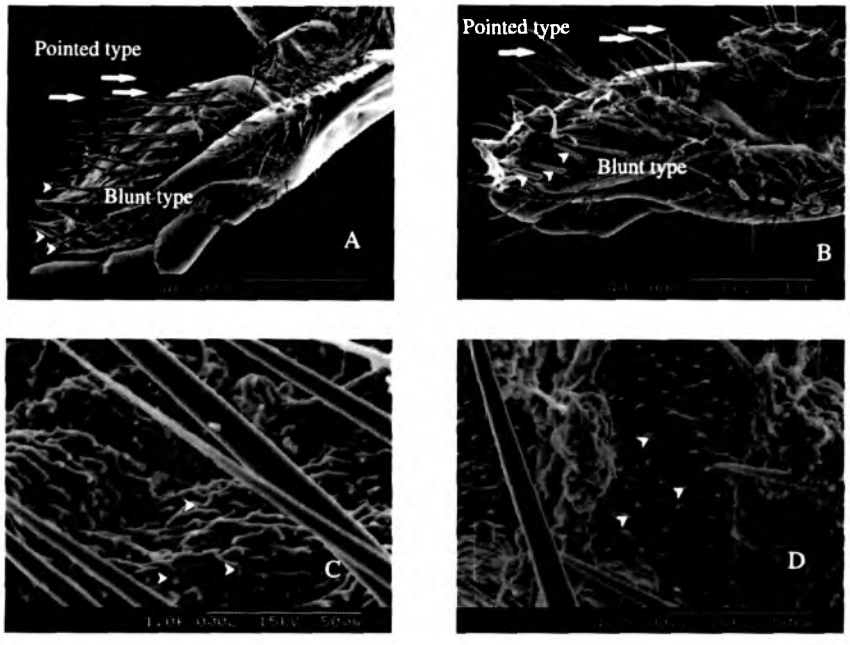
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Sreekumar and Prabhu (1988) by providing steam-sterilized cowdung as the medium and food. All the instruments used for ganglionectomy of larvae were autoclaved and kept in the absolute alcohol till used. Before surgery, the larvae were mildly ether-anesthetized and the head capsule was wiped with a cotton swab wet with absolute alcohol. For preventing excessive loss of haemolymph during surgery, the anterior thoracic region above the second spiracle was ligated using cotton thread. Then a "V" cut was made on the head capsule of the larva. The "V" flap thus made was lifted to expose the frontal ganglion. Using a pair of forceps, frontal ganglion was removed carefully and after applying a 1:1 mixture of ampicillin and phenyl thiourea, the wound was sealed using molten paraffin wax. The sham-operated controls were maintained after employing the same manipulation but without removing the ganglion. After 24 hours, the larvae were allowed to feed on 50 g rice powder wet with 10 ml water. The effect of frontal ganglionectomy on feeding was monitored by examining the gut for the presence of fresh food and recording body weight and weight and number of faecal pellets voided by the larvae. The maxillae of operated and sham operated larvae after 48 hours were removed and the morphological deformities were studied with scanning electron micrography following the method of Rekha and Sreekumar (2004)

The data obtained were analysed by student's "*t*" distribution. Mean and standard deviations were computed for the raw data ($n = 8$).

The stomatogastric nervous system plays an important role as accessory nervous system in the regulation of feeding in insects. In *Oryctes rhinoceros*, the frontal ganglion of stomatogastric nervous system has an important role in regulating gut motility and also motility of the mouth parts. The frontal ganglion appears as an intact pear shaped structure with apex pointing posteriorly, just in front of the brain above the esophagus. The frontal ganglion gives out a single frontal nerve, which passes to clypeus and also innervates mouthparts. The present study reveals that frontal ganglionectomy causes immediate cessation of feeding in the larvae of *Oryctes rhinoceros*. The gut of frontal ganglionectomised larvae showed no sign of fresh food whereas in sham-operated larvae the gut was full of food after 48 hours of surgery. Orlov (1924) observed that the peristalsis of the esophagus stops immediately after frontal ganglionectomy in *Oryctes nasicornis* larva. The cessation of feeding in the operated larva can be due to the arrest of foregut peristalsis. As a result of feeding inhibition, the operated larvae showed reduction in body weight (Table 1). The frontal ganglionectomy also resulted in significant reduction in the number and weight of the faecal pellets voided (Table 1). Similar effects were recorded earlier in *Schisostera gregaria* (Room, 1968), desert locusts (Hill *et al.*, 1966) and *Manduca sexta* (Griss *et al.*, 1991). In *Oryctes rhinoceros*, frontal ganglion has a critical role in feeding and moulting as reported in other insect Orders (Ayali, 2004). Mortality among the frontal ganglionectomised larvae was found to be 100%, 7 days after frontal ganglionectomy. Most of the larvae died with the onset of pupation. Ayali and Zilberstein (2004) have reported the same effects consequent to frontal ganglion ablation in fifth instar larval locusts, 48 hours before imaginal moult, recording 100% mortality. They have further



FIGURES 1. Scanning Electron Micrographs showing morphological alterations after frontal ganglionectomy in Oryctes rhinoceros larvae. A: maxilla of sham-operated larvae of Oryctes rhinoceros showing morphology of trichoid sensilla. B: maxilla of frontal ganglionectomised Oryctes rhinoceros showing changes in morphology of trichoid sensilla. C: inner stipe of maxilla of sham-operated larvae of Oryctes rhinoceros showing intact basiconic sensilla. D: inner stipe of maxilla of frontal ganglionectomised Oryctes rhinoceros showing degenerated basiconic sensilla.

observed that in the event of successful ecdysis the locusts failed to cast the old cuticle and died during the moult.

The scanning elactronmicrographs of maxilla reveal that, in the larvae of *Oryctes rhinoceros*, the morphology and number of sensilla are affected by the removal of frontal ganglion. These changes were mainly noticed in the sensilla responding to chemo-mechanical stimulus like trichoid and basiconic sensilla. The trichoid and basiconic sensilla can be distinguished into pointed and blunt types. In ganglionectomised larvae, the blunt type trichoid sensilla were found to be stouter and shorter than that in sham operated larvae (Fig. 1A and B). Some of the degenerated pointed type trichoid sensilla resembled the blunt ones. The pointed types are supposed to have chemo-mechanosensory function whereas the blunt ones may have only a chemosensory role. The present study also showed a 50% reduction in the number of basiconic sensilla in frontal ganglionectomised larvae compared to sham-operated larvae (Fig. 1C and D). Moreover, as in the case of trichoid sensilla, the basiconic sensilla in frontal ganglionectomised larvae also showed degenerative changes. The basiconic sensilla are

TABLE 1. Effect of frontal ganglionectomy on the third instar larvae of *Oryctes rhinoceros*

Parameter evaluated	Treatment	Days after surgery							
		0	1	2	3	4	5	6	7
Body Weight (g)	Sham-operated	11.6 ± 0.179	11.24 ± 0.173	11.492 ± 0.170	11.687 ± 0.132	11.897 ± 0.141	12.104 ± 0.182	12.282 ± 0.206	12.458 ± 0.203
	Frontal ganglionectomised	11.644 ± 0.271	10.92 ± 0.178	10.57 ± 0.164	10.2 ± 0.132	9.5* ± 0.142	9.15* ± 0.1770	8.5* ± 0.126	
Weight of faecal pellets (g)	Sham-operated		0.687 ± 0.0369	0.608 ± 0.0538	0.705 ± 0.011	0.818 ± 0.017	0.8075 ± 0.0197	0.802 ± 0.0259	0.964 ± 0.102
	Frontal ganglionectomised		0.121* ± 0082	0.105* ± 0.0160	0.097* ± 0.013	0.067* ± 0.012	0.033* ± 0.0192	0	
Number of faecal pellets	Sham-operated		13.166 ± 1.3291	11 ± 1.26	11.83 ± 0.83	14 ± 1.41	17 ± 1.41	16 ± 0.98	20 ± 1.2
	Frontal ganglionectomised		4* ± 0.89	2.8* ± 0.752	2.5* ± 0.542	1.8* ± 0.402	1* ± 0	0	

Each value represents mean of 8 observations ± SD

*Significance of values = 0.05 level.

associated with gustatory reception. These degenerative changes in the morphology of trichoid basiconic sensilla in frontal ganglionectomised larvae may cause weakened chemosensory input from maxillary sensory system. A rather good correlation between morphology and function has been demonstrated in insect sensilla by Steinbrecht (1998). The frontal ganglion act as a modulatory centre for impulses from various mouth parts and hence frontal ganglionectomy can block or weaken chemostimulatory input from the mouthparts causing cessation of feeding.

Hence to conclude, the present study demonstrates that in *Oryctes rhinoceros* larvae, removal of frontal ganglion significantly reduces feeding by affecting the foregut peristaltic movement frequency and also alters the morphology and number of chemosensory sensilla as well.

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Food preference of *Mylabris phalerata* Pallas (Meloidae: Coleoptera) in field conditions

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ABSTRACT: Feeding nature of *Mylabris phalerata* (Pallas), the most widely and endemic species of blister beetle (Meloidae) of India is presented here. The study was conducted in field conditions to observe its food preference. These insects in general get attracted to brightly coloured flowers and feed on them. Their food preference depend on a wide range of flowers of different host plants, among them *Ipomoea carnea* Jace. stands as a first priority of choice. © 2011 Association for Advancement of Entomology

KEYWORDS: *Mylabris*, food preference, *Ipomoea*

Blister beetles belonging to the family Meloidae is having many important biological parameters. On account of their complex and remarkable life histories of hypermetamorphosis (Richards and Davies, 1977) they are regarded as an important tool for fundamental as well as applied research. Some of the genus of this family Meloidae such as *Mylabris* have economic importance, and is the source of cantharidin (Balduf, 1935; Newport, 1851). Shukla and Upadhyaya (1971) reported that *Mylabris pustulata* Thunb an overlapping species of *Mylabris phalerata* (Pallas) preferred *Hibiscus rosa-scinensis* flower due to strong chemical attractant and stimulants present in this plant. But few studies were conducted so far in India on food preference of this insect in the field condition.

An outskirt area of Jhargram town under Dist. West Midnapore, about 155 km from Kolkata, West Bengal, India was selected for the study. The insects were observed in the field condition (50 mt. × 50 mt. quadrat) which was a marshy land and its soil was alluvial to sandy in nature. This area was selected due to the breeding site of the insects and on the availability of their host plants. The study was conducted during the month of July to September due to their occurrence and reproductive cycle. Observations were done every alternate day in the morning (7 AM to 8 AM). The minimum and maximum temperature were recorded at the time of observations in the field ranged between 27°C to 32°C, the humidity ranged between 60% to 95% and rainfall between

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TABLE 1. Food preference of *Mylabris phalerata*

Food plants (Family)	Common name	No. of insects observed	No. in percentage
Ipomoea carnea Jace. (Convolvulaceae)	Dholkolmi	328	88.41
Hibiscus rosa-sinensis Linn. (Malvaceae)	Jaba	25	6.74
Canna indica L. (Cannaceae)	Kalabati	11	2.96
Luffa acutangula Roxb. (Cucurbitaceae)	Jhinga	5	1.35
Lantana camara Linn. (Verbenaceae)	Kutus	1	0.27
Croton bonplantianum. (Euphorbiaceae)	Bontulsi	1	0.27

2 mm to 28 mm. The insects were observed while they were busy in feeding the floral parts without disturbing them.

The study revealed that among the host plants, *Ipomoea carnea* Jace. stands as a first choice in terms of food preference almost showing 88.41% (Table 1). The insect never spared a single flower, even an un-bloomed bud. These insects were voracious feeder of floral parts. They started feeding flowers from early in the morning. In a single flower of *Ipomoea carnea* two to three adults were seen to be feeding sometimes. They started cutting the upper rim of the petals and gradually got inside the bell of tubular shaped flower to feed on its reproductive part. In general they avoided the direct sun and when temperature rose they often took shelter below the leaf shed. These insects were also seen to be feeding under drizzle and some of them were also observed to be enjoying the rain in a motionless manner.

The next preferred food plants were *Hibiscus rosa-sinensis* Linn. (6.74%), followed by *Canna indica* L. (2.96%) and *Luffa acutangula* Roxb. (1.35%). They were found to nibble the flower of *Croton bonplantianum* and *Lantana camara* Linn. Xu *et al.* (2004) has reported that *Luffa cylindrical* was a preferred food of this insect. But present study revealed that locally available *Luffa acutangula* was only taken on selective basis. The study also revealed that *M. phalerata* mainly fed on the colourful petals and reproductive parts of food plants. The appearance of this insect was first observed on *Canna* flower in late June, as soon as the flowers of *Ipomoea* appeared, the adult beetles switched from *Canna* to their preferred type that was *Ipomoea*. Leaving all the flowers, these insects moved towards *Ipomoea* also probably due to its brilliant colour as well as due to their different taste. Moreover, these insects were found to eat the floral parts of *Ipomoea* in huge number due to easy availability of this flower during their reproductive period at that particular locality.

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Insecticide resistance against fenthion in *Culex quinquefasciatus* Say larvae of Delhi and National Capital Region

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ABSTRACT: Fenthion is being used as a larvicide in the National Vector Borne Disease Control Programme (NVBDCP) for the last two decades. The larvae of *Culex quinquefasciatus* collected from Delhi and National Capital Region (NCR) were exposed to the diagnostic dose (0.05 ppm) and also to a series of concentrations of fenthion ranging from 0.05 to 0.3 ppm as per the standard WHO susceptibility test kit method under laboratory conditions. The larvae from different parts of Delhi were exposed to the diagnostic dose of fenthion and recorded the larval mortality ranged from 3.0 to 24.0 percent and larvae from the NCR area the mortality ranged from 30.0 to 58.0 percent. In Delhi area, the average LC_{50} values recorded was 0.09 ppm (0.06 to 0.14 ppm) and in the NCR area 0.05 ppm (0.03 to 0.07 ppm). In Delhi area, the average LC_{90} values recorded was 0.18 ppm (0.13 to 0.29 ppm) and in the NCR area 0.14 ppm (0.12 to 0.16 ppm). The study revealed that the larvae have developed resistance against fenthion in all parts of Delhi and also in NCR area. It is estimated that in Delhi area 3.6 fold and NCR area 2.8 fold build up of insecticide resistance in the larvae of *Cx. quinquefasciatus* against fenthion.
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KEYWORDS: fenthion, larvicide, resistance, *Culex quinquefasciatus*, Delhi and National Capital Region

INTRODUCTION

Filariasis is the second leading cause of permanent and long-term disability in the world. Approximately 170 million people in the tropical and subtropical areas of Southeast Asia, South America, Africa and the Islands of the Pacific are affected by this debilitating parasitic disease. More than 1.3 billion people in 81 countries

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worldwide are threatened by lymphatic filariasis, over 120 million people are currently infected and about 40 million disfigured and incapacitated by the disease. Lymphatic filariasis afflicts over 25 million men with genital disease and over 15 million people with lymphoedema. In India, filariasis is endemic in 250 districts in 20 States/Union Territories with about 553 million people at risk of infection. The Government of India has accorded a high priority for elimination of this infection through mass drug administration (MDA) programme.

Cx. quinquefasciatus, vector of filariasis and a nuisance mosquito in most of towns and cities of India. The high density of this species mainly related with the disposal of wastewater and sanitation (Batra *et al.*, 1995). Chemical control is the main method of vector control adopted in urban area to keep the density of mosquitoes under control. In urban area, fenthion is being used as a larvicide in polluted water to control the larvae of *Cx. quinquefasciatus* since last two decades (National Malaria Eradication Programme, 1995). Continuous use of any insecticide over a period of time will result in the precipitation of resistance in the target species (Brown and Pal, 1971). Development of resistance against fenthion in the larvae of *Cx. quinquefasciatus* was reported world over including India (WHO, 1992). However, no data is available on the susceptibility status of larvae of *Cx. quinquefasciatus* of Delhi and National Capital Region (NCR) against various insecticides currently being used in the public health programme. Hence a study was carried out to assess the current insecticide susceptibility status of larvae of *Cx. quinquefasciatus* against fenthion.

MATERIALS AND METHODS

National Capital Territory (NCT) of Delhi is divided into 14 zones and the larvae of *Cx. quinquefasciatus* mosquito were collected from different localities of each zone. Broadly, these zones are grouped into five mega zones namely East, West, North and South and Central zones. Delhi is surrounded by two states, Uttar Pradesh and Haryana and mosquito larval collections were also made from different localities of NCR (Fig. 1). The study was carried out during the period from March to May, 2010. Larvae were collected from stagnant water collections either due to faulty drainage system or rain water. The mosquito larvae collected from different breeding sites were transported to the laboratory for identification of the species. The larvae of *Cx. quiquefasciatus* were selected out and washed in tap water to remove debris and kept for observation for a period of 2 hours to weed out the dead and moribund larvae. Early fourth instar larvae of *Cx. quinquefasciatus* were used for carrying out the insecticide susceptibility test. The larvae were exposed to the diagnostic dose of fenthion (0.05 ppm) and to a series of doses prepared from the technical grade of fenthion (82.5% w/w) obtained from Ficom Organic Ltd., Mumbai, India. The test was performed by exposing the twenty early 4th instar larvae in 500 ml beaker containing required dose of larvicide prepared in 250 ml of water. For each dose, three replicates and one control was kept. Each test was repeated and the mortality count was made after 24 hours of exposure period. The tests showing more than 20% mortality in control were discarded and repeated. In cases the control mortality ranged between



FIGURES 1. Map of Delhi and NCR showing the location of mosquito larval collection sites for the testing

5 and 20%, the corrected mortality was calculated using Abbott's formula (WHO 1981a). The susceptibility status was 'Resistant' when mortality rate recorded was less than 80%, and 'Tolerant' while 80–98%. Probit analysis was carried out to determine the LC_{50}, LC_{90}, heterogeneity and resistance in fold (Abbott 1925).

RESULTS

Results of the susceptibility tests carried out with the larvae of *Cx. quinquefasciatus* against diagnostic dose of fenthion is given in Table 1. The percent larval mortality observed varied from 3.0 to 24.0% in the larvae collected from different zones of Delhi, while mortality varied from 30.0 to 58.0% in the larvae collected from NCR area of Delhi. The tests revealed that 100% larval mortality was obtained at the doses ranged from 0.25 to 0.3 ppm in the larva collected from different zones of Delhi and 0.15 to 0.2 ppm from the NCR area.

TABLE 1. Insecticide susceptibility status of larvae of *Culex quinquefasciatus* of Delhi and National Capital Region against the diagnostic dose of fenthion

Area	No. of larvae exposed	No. of larvae dead (After 24 hrs. of exposure)	Percent mortality	Susceptibility/ Resistant status
Delhi				
North Delhi	720	22	3.0	R*
South Delhi	840	25	3.0	R
West Delhi	1080	44	4.0	R
East Delhi	720	72	10.0	R
Central Delhi	1320	317	24.0	R
National Capital Region				
Haryana	720	418	58.0	R
Uttar Pradesh	360	108	30.0	R

*R — Resistance

TABLE 2. Dose mortality response of larvae of *Culex quinquefasciatus* of Delhi and National Capital Region against fenthion.

Area	LC_{50} values	LC_{90} values	Heterogeneity (χ^2) df*	Resistance (In fold)
Delhi				
North Delhi	0.10	0.17	43.49(5)	3.4
South Delhi	0.14	0.29	39.47(6)	5.8
West Delhi	0.07	0.13	36.95(6)	2.6
East Delhi	0.08	0.16	11.90(6)	3.2
Central Delhi	0.06	0.14	36.48(6)	2.8
National Capital Region				
Haryana	0.03	0.12	23.96(5)	2.3
Uttar Pradesh	0.07	0.16	34.56(5)	3.2

*df — Degrees of freedom

Results of the probit analysis to determine the LC_{50}, LC_{90}, heterogeneity and resistance build up in fold is given Table 2. The LC_{50} values estimated against fenthion was ranged from 0.06 to 0.14 ppm (Average −0.09) in the larvae tested from Delhi area, while 0.03 to 0.07 ppm (Average −0.05) in the larva collected from NCR area. The LC_{90} values estimated for the larva collected from Delhi area ranged from 0.13 to 0.29 ppm (Average 0.18) and from NCR area ranged from 0.12 to 0.16 ppm (Average −0.14). The heterogeneity factor calculated was varied from 11.90 to 43.49 in the larvae of Delhi area while 23.96 to 34.56 from the NCR area (Table 2). In Delhi area the build up of development of resistance against fenthion was ranged from 2.6 to 5.8 times higher than the diagnostic dose while 2.3 to 3.2 times fold in the larvae collected from NCR area (Table 2).

DISCUSSIONS

About 25 countries including India have already reported the development of resistance in the larvae of *Cx. quinquefasciatus* mosquitoes against organophosphate compound (WHO, 1992). Resistance to fenthion in the larval stage has earlier been reported from Japan and Korea (Brown and Pal, 1971). In India resistance against organophosphate compound in *Cx. quinquefasciatus* have been reported from various parts of the country (Pillai, 1996). Earlier studies carried out in Delhi area revealed the possible development of resistance against fenthion in the larva of *Cx. quinquefasciatus* (Mittal, 1999). There are several reports that the larvae of *Cx. quinquefasciatus* have developed resistance against temephos, another organophosphate compound in different parts of the country. Limited field trials were also carried out with temephos 50% EC in Rajamundary town (Andhara Pradesh) revealed that even after the use of four fold increased dosage of temephos could not yield the desired mortality in the larvae of *Cx. quinquefasciatus* larvae (Krishna Rao *et al.*, 1989).

The present study confirms the development of resistance in the larvae of *Cx. quinquefasciatus* mosquitoes against fenthion as the percent mortality recorded against the diagnostic dose of fenthion was ranged from 3.0 to 24.0 and 30.0 to 58.0 percent from Delhi and NCR area respectively. The LC_{90} values for fenthion recorded from different parts of Delhi was ranged from 0.13 to 0.29 ppm and from NCR areas ranged from 0.12 to 0.16 ppm. The average build up of resistance in different parts of Delhi and NCR area in the larvae of *Cx. quinquefasciatus* recorded to be 3.6 and 2.9 fold respectively.

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Drosophila hegdii, a new species of *Drosophila* (Diptera: Drosophilidae) from Lumami (Nagaland: India)

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ABSTRACT: New species *Drosophila hegdii*, member of the montium subgroup of *melanogaster* species group collected from sub-tropical, evergreen forest in Lumami, District Zunheboto, State Nagaland, India is described. The taxonomic status and relationship are discussed. © 2011 Association for Advancement of Entomology

KEYWORDS: *Drosophila*, Drosophilidae, *Sophophora*, new species, Lumami, Nagaland, India

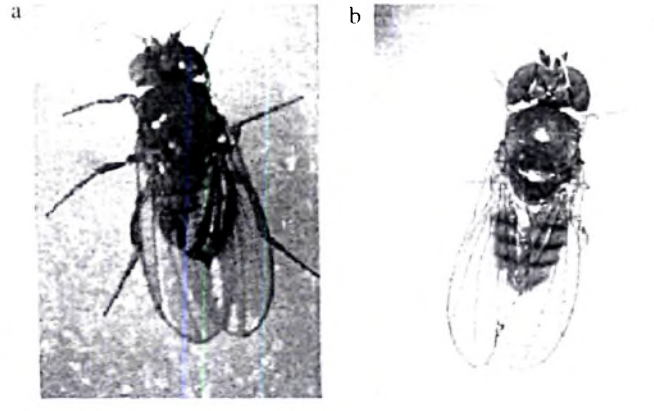
The Indian subcontinent with its vast array of vegetation and climatic conditions harbors many species of *Drosophila*. During the last few decades several investigators have surveyed the *Drosophila* fauna in various parts of South India (Reddy and Krishnamurthy, 1968, 1977; Sajjan and Krishnamurthy, 1975; Gowda, 1979; Muniyappa *et al*., 1981; Gai, 1985; Hegde *et al*., 1989). Little work is done on *Drosophila* fauna of north east India (Singh and Gupta, 1977; Dwivedi and Gupta, 1979; Gupta and Singh, 1979; Singh and Gupta, 1980).

The present survey of *Drosophila* was undertaken in Lumami of Nagaland, a sub Himalayan hilly state of north east India which is situated at 94.28° E Longitude and 26.13° N Latitude, having an altitude of 940 m above sea level. In Lumami climate is pleasant, generally cool in winter and warm in summer; temperature ranges from 17°C to 30°C and average annual rainfall is about 200 cm. The torrential monsoon rain is an integral feature of the weather. Heavy rainfall during the monsoon favours the growth of thick forest, fruit bearing trees, providing favourable natural habitats for the colonization by the members of the genus *Drosophila*. Collections were made by trapping the flies on banana baits.

A new species collected in the locality of Lumami belonging to *melanogaster* species group of the subgenus *Sophophora* is described here.

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FIGURES 1. Female and male forms of *Drosophila hegdii*.

Male and female (Figure 1)

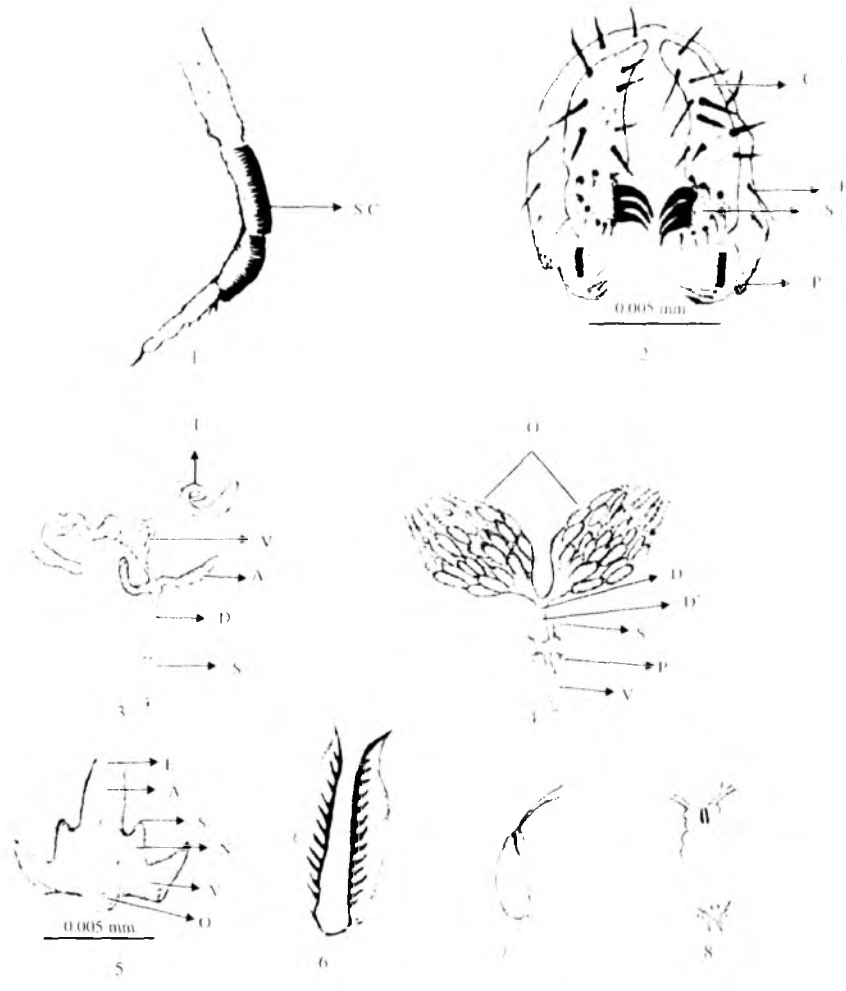
Light brown flies. Body length for female is 100 mm and for male is 97 mm.

Head

Arista with 4 branches above, 3 below, plus the terminal fork. Antenna dark, basal segment of the antenna bears a pair of dark bristles. Vibrissae with two anterior and two posterior bristles. In between the anterior and posterior bristles are 10–12 small bristles. Palp with a large and many small bristles. Anterior orbital proclinate, median orbital half the size of anterior orbital, posterior equal to anterior. Anterior verticles direct inward, posterior convergent. Ocular triangle with a pair of dark bristles, eyes red.

Thorax

Mid to dark brown, acrosticals in 8 regular rows, dorsocentrals convergent, anterior dorsocentrals are shorter than the posterior- approximately $2/3^{rd}$ the length of the posterior, anterior scutellar convergent, posterior scutellar convergent and crossed. Both anterior and posterior scutellars are of equal length. Two humerals, upper humerals half the length of the lower, posterior allars longer than anterior. Notopleurals and stenopleurals are of equal length. Notopleural and supra allars are of equal length. There are about 2–3 smaller bristles along the anterior and posterior stenopleurals. Halters translucent.



FIGURES 2. (1) S.C sex comb in first & second tarsal segment; (2) Periphallic Organ E – Epandrium; P – Primary Claspers; S – Secondary Claspers; C – Anal Cercus; (3) Male reproductive system: T – Testis; V – Vasa deferentia; A – Paragonia; D – Anterior ejaculatory duct S – Sperm pump; (4) Female reproductive system: O – Ovaries; D – Oviduct; D' – Common oviducts; S – Spermathecae; P – Paravaria; V – Vagina; (5) Phallic Organ: E – Edeagus; A – Anterior gonopophysis; S – Spines; N – Novasternum; O – Basal apodeme; V – Ventral fragm; (6) Egg guide; (7) Egg; (8) Pupa.

Wings

Transparent, wing length of male is 97 mm and female is 100 mm. Wing indices are calculated following the formula of Okada (1956) and presented in the Table 1.

Legs [Figure 2(1)]

Sex comb present in male on first and second tarsal segment. First tarsal consists of about 25–27 teeth and second tarsal consists of 16 teeth. Teeth are uniform and slightly curved.

Abdomen of male and female

First four tergites of male are shiny yellow-brown with broad dark apical band, last two segments are completely black. Tergites of Female are shiny dark brown with broad darker apical bands.

Internal characters

Female reproductive parts consist of ovarioles with 5–6 ventral receptacles that are transparent with 2–3 coils, spermetheca roundish colourless [Figure 2(4)]. Male testis is short showing 2–3 coils and light yellow in colour, paragonia spherical transparent [Figure 2(3)].

Periphallic organ [Figure 2(2)]

Ependrium broad, dorsally and laterally. Primary and secondary claspers present, primary claspers with a lateral row of about 5 teeth and a ventral medial cluster of teeth one elongated, toe with 3–4 bristles; secondary claspers oval, partially separated from anal plate with 3 black teeth, two are prominent and one is rudiment and about 1–8 small bristles along the ventral lateral and dorsal borders. Circus rounded on the outer side and slightly curved on inner side and with about 25 long and short bristles.

Phallic organ [Figure 2(5)]

Adeagus and anterior gonopophysis not fused. Anterior gonophysis protrude dorsally. Novasternum with prominent median convexity of variable thickness bearing a pair of spines, ventral fragma broad and concave, basal apodeme is thick and short.

Egg guide [Figure 2(6)]

Brown in colour with about 9–10 marginal and 1–2 discal teeth at the tip, teeth are dark in colour.

Egg [Figure 2(7)]

White in colour with two filaments present at the anterior.

TABLE 1. Wing indices of *Drosophila hegdii*
(Mean value of 10 flies)

Sex	Costal index	4V index	4C index	5X index
Male	2.69	1.15	2.55	2.62
Female	2.81	1.1	2.75	2.5

Pupa [Figure 2(8)]

Yellow with 9–10 spiracle filaments. At the posterior end there are 3 pairs of projections- one pair is lateral, second pair is ventral and third pair is dorsal

Holotype

Male: India, Nagaland, Lumami, 14.xi.11 Coll. Bovito Achumi and Sarat Chandra Yenisetti.

Deposited in the *Drosophila* vivarium of Department of Zoology, University of Mysore,

Manasagangotri, Mysore 570006, India.

Allotype

Female: Same as above.

Paratype

5♂♂ and 5♀♀, India, Nagaland, Lumami; Coll. Bovito Achumi and Sarat Chandra Yenisetti.

Taxonomic status

The nature of the banding pattern of the abdominal tergites, the presence of 2 egg filaments and the puparia warrant its inclusion in the subgenus *Sophophora*. The presence of long ventral receptacle, coiled testis, convergent scutellars and two pairs of malphigian tubules qualify its inclusion in the *melanogaster* species group (Patterson and Stone, 1952). Further the presence of sex comb extending beyond the tips of the tarsal joint, the presence of primary claspers and secondary claspers with curved black teeth permit its inclusion in the montium sub group (Bock and Wheeler, 1972).

It was found that the new species resembles *D. vulcana* in the general colouration of the body, but differed in other morphological characters such as the number of teeth in sex-combs, the nature of arrangement of teeth in the sex comb, the prominent teeth, sex comb extending beyond the tips of the tarsal joints, the prominent teeth in the secondary claspers, number of rows of acrostical hairs, wing indices, periphallic and phallic organ. In addition the new species differed from other known species of montium sub group in characters such as the number of teeth in sex comb and abdominal banding pattern

Hence it deserved the status of a new species in the montium sub-group. This species is named as *Drosophila hegdii* in honour of Prof. S.N. Hegde for his contribution to *Drosophila* taxonomy.

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